STUDIES

FROM

THE ROCKEFELLER INSTITUT

FOR MEDICAL RESEARCH

REPRINTS

VOLUME XII

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STUDIES UPON A TRANSPLANTABLE RAT TUMOR.

ORIGINALLY REGARDED AS A SARCOMA; PROBABLY A TERATOMA FROM WHICH AN ADENO-CARCINOMA DEVELOPED.

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PLATES I–XVI.

By far the greater number of studies of transplantable tumors have been made upon mice, in which species tumors not infrequently arise spontaneously. These tumors consist for the most part of the epithelial neoplasms which take their origin from the mammary glands, as was originally pointed out by Livingood, and present the histological appearances of adenoma and adeno-carcinoma, the dividing line between the two being indefinite. Other types of tumors have been found very rarely in mice. Livingood described a sebaceous adenoma; Ehrlich, a chondroma; Haaland and Tyzzer, lympho-sarcomata; Bashford, Murray and Haaland, epidermal cancer; and, more recently, Jobling has met with an instance of what appeared to be Hodgkins's disease, and two examples of papillary cystoma of the ovary. In several instances, namely, in the cases reported by Ehrlich and Apolant, Leo Loeb, Bashford and Liepman, adenomata have been described which through hypertrophy of the stroma and elimination of the epithelial elements have changed slowly or more quickly into spindle-cell sarcomata. Once the sarcomatous condition has become established, it has persisted through many transplantation generations.

The transplantable mouse tumors have been studied with great profit, and the high degree of success which has attended these studies and the considerable advance made in uncovering the biological conditions underlying tumor growth have resulted in large part from the happy circumstance that a fair percentage of spontaneous tumors of mice are transplantable into individuals of the same race

or species, and also from the facts of the small size, rapid maturation and brief life history of the mouse, which favor the making of observations on a large and sufficient scale to yield data of scientific value.

The number of tumors of rats, which have been studied in a manner similar to the tumors of mice, is small, in spite of the fact that rat tumors had been transplanted successfully before mouse tumors. Moreover, the variety of rat tumors thus far transplanted exceeds the variety of transplantable mouse tumors, for while nearly all the mouse tumors thus far described have been epithelial neoplasms derived from the mammary gland of the female mouse, far more sarcomata have been encountered in rats than epithelial tumors, and both have sprung from diverse parts of the body.

In 1889 Hanau transferred successfully an epidermal cancer of the rat through two generations. The only previously successful experiments had been made by Novinski and by Wehr. In 1876 Novinski implanted a cancer of the nose of a dog into two others, in one of which a lymphatic gland metastasis appeared; and in 1889 Wehr inoculated successfully the so-called carcinoma of the penis of the dog. As we now know, the latter tumor is not a cancer, but probably a round cell sarcoma.

The instances in which adenomatous tumors of the mammary gland of the rat of a transplantable nature have been recorded are very few, the chief one consisting of the instance reported by Michaelis and Lewin. It is noteworthy that this tumor lost, after a small number of passages, its adenomatous and took on a sarcomatous character. We wish to add another word to the subject of the discrepancy in the histological structure and place of origin of mouse and rat tumors. The records of the spontaneous tumors of animals are very imperfect and take account of little more than the transplantable examples. We know from our own experience that simple adenomatous and fibromatous tumors arise not so very infrequently from the mammary gland of the female rat, although they have not up to the present time been transplanted successfully. We have records of three such examples of benign fibro-adenomatous neoplasms, as well as of a lipoma of the subcutaneous tissues.

We have brought together in brief form the recorded instances

of transplantable rat tumors.　As stated, the first report was by Hanau (1889), who observed three instances of epithelial cancer among some white rats raised in the laboratory.　The cancer affected the skin of the external genitals.　The transplanted tumor arose in the region of the vulva, and produced inguinal and axillary gland metastases.　Portions of the latter were used for transplantation, and the secondary nodules showed the histological structure of the original tumor.　In one animal in which the inoculation was into the scrotum, there were multiple peritoneal nodules, and masses within the diaphragm, and one mass in the posterior mediastinum.

In 1890 von Eiselsberg reported an instance of a spindle-cell sarcoma of a mixed gray and white rat, which arose in the periosteum of the scapula.　It was transplanted successfully into the peritoneal cavity of another rat.

In 1892 Firket reported an example of spindle-cell sarcoma of the peritoneal cavity of a white rat.　A series of successful transplantations was made, and the original histological structure of the tumor was always reproduced.　An effort was made to implant the tumor upon guinea-pigs, but unsuccessfully.

In 1898 Velich transplanted a sub-periosteal spindle-cell sarcoma of the thigh of the white rat through several generations.　One series of rats was inoculated into suppurating sinuses without result. Another was inoculated with blood from the field of incision of the tumor, also without result.　In one rat the grafted tumor penetrated into the depth of the tissues, compressed the spinal cord, and produced motor paralysis.　Two guinea-pigs were inoculated without result.　Rats inoculated with fluid derived from the tumor, but free from visible particles, yielded no tumors.　Suspension of tumor particles in salt solution before injection into the peritoneal cavity did not prevent tumor formation.　From the intra-peritoneal masses subcutaneous tumors were developed in other rats.　A second graft was successfully implanted in a rat in which a growing tumor already existed.　Pieces of tumor kept sterile and outside the body for twenty-four hours and for three days before inoculation produced tumors, but after four days none developed.　A progressive decrease in the energy of tumor growth from the first to the ninth generation was observed, and no further transplantations were

obtained after the ninth generation.   An instance of spontaneous inoculation of the jaw of a rat was observed in an animal which gnawed the tumor of a rat kept in the same cage.   By wounding a rat at the back of one of the canine teeth and allowing it to gnaw a tumor, a nodule was made to develop in the wound, and since the tumor gnawed was situated in the subcutaneous tissue of the same rat, the result is an illustration of the development of a second tumor in an animal possessing a growing tumor.

Leo Loeb in 1901 transplanted through many generations a cystic sarcoma of the thyroid gland of the white rat, which was inoculable under the skin and in the abdominal cavity and by means of the cystic fluid derived from the tumors.   The fat of the implanted fragments was studied.   Of the grafts many of the cells degenerated, but a number retained a normal appearance and mitoses were observed in the periphery as early as fifteen or twenty days after implantation.   Of these some probably belonged to the tumor cells.   In from five to eight days a capsule of connective tissue had surrounded the graft.   The center of the graft at this time showed shrunken cells with pyknotic nuclei and blood vessels with desquamated epithelium, but not all the cells were necrotic.   Mitoses were visible as late as the twelfth to the fifteenth day, at about which time the tumor cells invaded the capsule.   In order to ascertain the demarcation between host and tumor cells the grafts were enclosed in gauze sacs, and in spite of suppuration about many of the sacs, a part of the grafts developed, but the result was not definitive.   It was noted that when the graft became infected its growth was retarded.   After arrest of growth, excision of a part sometimes led to renewal of development, and replantation of the excised part to another part of the body was sometimes followed by growth of the new graft.   In some cases in which the excision failed to awaken growth in the original tumor the graft derived from it, implanted in another animal, yielded a tumor.   Local metastases were produced, but systemic metastases did not develop even when the tumor invaded a blood vessel.   The examples of contact inoculation were most pronounced from intra-peritoneal implantations, and the separate nodules even penetrated the diaphragm and projected into the pleural cavity.   The histology remained fixed during fifteen months

of transplantation. Attempts to implant the tumor into guinea-pigs, hens and mice failed. In guinea-pigs, at first mitotic division of the tumor cells occurred, but the grafts did not increase in size and the emigration of leucocytes was more abundant than about the grafts in rats. The opinion is expressed that in the production of tumors by grafting the new tumor is derived from the cells of the graft and not from the cells of the host; and Loeb, therefore, views these tumor cells as resembling germ cells in their capacity for unlimited reproduction.

In 1902 Loeb described several more rat tumors. One was an adeno-carcinoma originating apparently in the pancreas and not transplantable. Another depended from the neck, originated in the thyroid gland and was divided unequally by a fibrous band into halves, one of which was an adeno-carcinoma and the other a cystic sarcoma. A single sarcomatous metastasis existed in the lung. The sarcoma was transplantable. A third rat showed an adenoma of the mammary gland. It was excised and fragments were implanted beneath the skin of the same animal and of other rats. A month later the fragment implanted in the original rat had increased to eight times its former size, when the animal gave birth to young, after which in a remarkably short time the transplanted tumor shrank in size. No growth took place in the other rats inoculated. A fourth tumor was a cystic sarcoma of the thyroid gland, which had produced local and inguinal lymphatic gland metastases. Portions of this tumor kept on ice for five days grew on transplantation, while other portions kept in the thermostat or at variable room temperature failed to grow. Loeb concluded, erroneously, as we now know, from these experiments, that two agencies are required to cause growth of the grafts—the tumor cells and a tumor-producing factor. The latter he believed did not lose power on exposure to temperatures of 2° to 4° C., while he believed it less certain that the sarcoma cells remain alive at those temperatures. He found that injections of tumor pulp suspended in salt solution and of filtrates through paper, asbestos and unglazed porcelain gave negative results. The tumor-producing factor was conceived to be rendered inactive by salt solution. Loeb considered that two factors were concerned in immunity to tumor grafts, one being a state of

resistance to the transplanted tumor cells and the other to the tumor-producing agency.

In 1903 Loeb described in a paper dealing with the endemic occurrence of tumors in animals still another example of cystic sarcoma of the thyroid gland of the rat.

Gaylord and Clowes in 1907 described three rats which developed tumors while being kept in cages previously employed by Loeb in his experiments with a transplantable sarcoma of the thyroid gland. In 1904 a rat was discovered showing a fibro-sarcoma of the abdominal wall and axillary region, and in 1905 two rats were found showing respectively fibro-sarcoma of the abdominal wall and spindle-cell sarcoma of the thyroid gland. The last tumor was transplantable.

In 1907 Jensen reported an instance of transplantable spindle-cell sarcoma of two gray-brown rats which had previously—and before the tumors developed—been inoculated with an acid-fast bacillus derived from a pseudotuberculous disease of cattle. Rat 1 showed nodules of varying size in the peritoneal cavity, and small nodules in the liver and lungs. The growth was infiltrative. Rat 2 showed small nodules in the lungs and none elsewhere. From Rat 1 inoculations were made into wild rats and rats obtained from Berlin, but without results, and into several brown and white rats of the laboratory stock, some of which developed the tumor. Later certain Berlin and London rats were inoculated with occasional success. Gray rats, however, were found refractory. The tumor from Rat 2 could also be transplanted to the laboratory stock, but not to the rats of foreign origin.[1]

In 1907 Michaelis and Lewin published their studies of a transplantable carcinoma of the rat which in later generations became converted into a spindle-cell sarcoma. The original tumor developed in the mammary gland. The first transplantations yielded about 50 per cent. of tumors and produced lung metastases. Some of the tumors underwent complete retrogression. It was noted that young rats were more subject to the implantations than old ones, and that the white, gray, mixed and black rats were all susceptible

[1] Professor Jensen kindly sent us living rats possessing tumors, which we have propagated successfully through many generations in American rats.

by subcutaneous and by intra-peritoneal inoculation. Even before the conversion of the tumor into sarcoma it had been noted that the microscopical characters of the growth varied considerably and sometimes presented appearances of pure adenoma; at other times, of alveolar and solid carcinoma; and at still other times, on account of the rich development of the stroma, of sarcoma. Moreover, there sometimes developed cystic spaces carrying papillary outgrowths, and the individual cells varied so greatly in respect to size, quality of protoplasm and nature of nucleus as to excite wonder that they could all arise from a single tumor. One tumor of the third generation is stated to have exhibited in places the appearances of typical cancroid. The overgrowth of stroma which eventually gave rise to a sarcoma began in the fifth generation and in the seventh generation the sarcoma had been perfected. By choosing the most rapidly growing strains, and by double inoculations at intervals of a few days, the number of successful implantations was made to reach 100 per cent. Rats with growing tumors could be successfully implanted with a second graft. Rats which had recovered spontaneously from growing tumors were resistant to the several types of this tumor, namely, the adenoma, epithelioma and sarcoma, and to Jensen's sarcoma. The percentage of spontaneous recoveries was about ten. The blood of the rat gave a considerable degree of immunity and a reciprocal immunity was produced between mice and rats by inoculating with the rat tumor, on the one hand, and mouse carcinoma, on the other.[2]

Apolant in 1908 reported very briefly upon twenty-four rat tumors which had been collected by Ehrlich's laboratory. They consisted of one fibro-lipoma, two fibromata, three adeno-fibromata, thirteen sarcomata, and five tumors which were designated mixed tumors of the "Flexner type."[3] He speaks also of two forms of rat sar-

[2] Dr. Lewin kindly sent us specimens of living rats with tumors of the sarcomatous variety, which we have propagated successfully in American rats through a number of generations.

[3] It is perhaps questionable, in view of the transformations through which the rat tumor we are describing passed, whether Apolant is correct in speaking of the tumor as a mixed one, and in giving it such a specific cognomen. If it is true that the tumor we are describing represents merely a type of mixed tumor, which is not uncommon in the rat, it would be quite significant, we think, par-

comata according to their microscopical structure.   One of these presented the characters of an ordinary spindle-cell sarcoma, whereas the other possessed the characters of a round cell sarcoma with lymph-adenoid structure.   The latter type was not homogeneous, but showed in some parts a cellular structure resembling that of the lymphatic glands, in which there was a reticulated tissue composed of spindle and branching cells, in the meshes of which lay the round cells, and in other parts a looser tissue composed essentially of the reticulated tissue.   Moreover, in still other places the structure approached the type of the spindle-cell sarcoma.   It is stated that this tumor possessed from the beginning a high degree of energy of proliferation, and yielded a high percentage of successful inoculations.   In the course of the implantations of this tumor it exhibited more and more the character of a spindle-cell sarcoma, until finally it became a pure tumor of this type.   The reverse process was observed in still another tumor, which, at the beginning, presented the usual characters of the spindle-cell sarcoma of rats, and gradually became transformed into a tumor presenting lymph-adenoid structure.

HISTORY OF THE TUMOR.

A full grown, white, male rat of the laboratory stock died spontaneously on January 5, 1906.   The autopsy performed soon after death revealed a tumor the size of a walnut attached to the left seminal vesicle and projecting into the abdominal cavity.   There were no adhesions to other viscera.   The tumor was approximately spherical in shape and was covered with peritoneum.   The surface was roughened by irregular granulations.   The consistence was firm and the mass cut with difficulty.   There were no visible metastases.   The cut surface of the tumor was not uniform; it was in general dead white in color, but certain areas were more opaque and of a yellowish tinge.

ticularly in view of the manner in which the highly organized type of tumor has been developed in our example. On the other hand, we are inclined to believe that the facts to be presented concerning the structure of our tumor with reference to the original moiety of adeno-carcinomatous structure will suffice to put it in a place quite by itself.

A small piece of the tumor was cautiously removed, cut into bits with sterile scissors, suspended in sterile normal salt solution and injected through a needle with a large bore into the abdominal cavity and beneath the skin of three white rats, the histories of which will be given later.

The remainder of the tumor was preserved for histological examination. In the course of the studies of the tumor carried out during the past few years the original mass has been subjected to repeated microscopical examinations.

In our first publications[4] regarding this tumor we described it as a polymorphous sarcoma. As the following description will show, the original tumor cannot really be brought into harmony with the morphology of any of the established types of sarcoma. It would appear certain now that the original tumor should not have been regarded as a single type. In the course of transplantation it has passed through several forms of structure corresponding with sarcoma and simple and adeno-carcinoma, and has come to be established in the last form. When the carcinomatous forms made their appearance the changes in the structure of the tumor were so remarkable, and apparently so abrupt, that the origin of the new elements from the previously perceived ones in the original tumor seemed exceedingly doubtful. Hence the original mass, which had been preserved, was subjected to reëxamination by sections made from all parts of it. The result was the discovery of certain structures of an epithelial character that pointed to the existence of an adenomatous moiety in the original tumor. These epithelial elements consisted in part of glandular structures of a normal type, and in part of such atypical formations as are seen in adeno-carcinoma. Certain of these glandular bodies of normal appearance seemed indeed to be participating directly in the production of

[4] Flexner and Jobling, Infiltrating and Metastasizing Sarcoma of the Rat, *Jour. of the American Med. Assoc.,* 1907, xlviii, 420; Infiltrierendes und metastasenbildendes Sarcom der Ratte, *Cent. f. allg. Path.,* 1907, xviii, p. 257; Remarks on and Exhibition of Specimens of a Metastasizing Sarcoma of the Rat, *Proc. of the Soc. for Exper. Biol. and Med.,* 1906–07, iv, 12; On Secondary Transplantation of a Sarcoma of the Rat, *ibid.,* 44; On the Promoting Influence of Heated Tumor Emulsions on Tumor Growth, *ibid.,* 156; Restraint and Promotion of Tumor Growth, *idem,* 1907–08, v, 16; Metaplasia and Metastasis of a Rat Tumor, *ibid.,* 52; Further Notes on a Rat Tumor, *ibid.,* 90.

the atypical formations indicative of carcinoma, thus indicating possibly the origin of the carcinomatous elements from several distinct foci.

It now appeared, therefore, as if the original growth within the seminal vesicle first consisted of a morphologically simple tumor made up of one kind of tissue, that by invasion of the inner or epithelial wall of the vesicle and by inclusion of certain epithelial structures became, as it were, infected with these epithelial cells, which upon removal from their normal relations and control assumed malignant properties. According to this view the epithelial moiety was inconspicuously present in the part of the tumor that was originally transplanted, and it gradually divested itself in the course of the transplantation of the prevailing simple tissue until it became itself dominant.

It may be questioned whether the part of the tumor originally described did not possess a greater complexity of structure than could properly be attributed to a so-called polymorphous sarcoma. Indeed, it was pointed out originally that the relation of cells and connective tissue within the original tumor was such as to permit its being described as imperfectly alveolar. The sections exhibited a connective tissue framework, varying in density, containing spindle cells and supporting other cells far richer in protoplasm. The latter cells occupied spaces of variable size and form, so that sometimes a single cell and sometimes several cells were thus enclosed. This relation of large cells to stroma was particularly well shown in sections stained by Mallory's aniline blue and fuchsin method.

The complexity of the original mass does not end here, for the inclusions at one point were such as to arouse the feeling that the tumor was, in fact, originally a teratoma or embryoma, and the subsequent transformations, first into a tumor resembling polymorphous sarcoma, and next into an adeno-carcinoma, represented changes of secondary nature.

HISTOLOGY OF THE ORIGINAL TUMOR.

Under the low power of the microscope the sections showed considerable diversity. Tissue taken from the free part of the mass, away from its attachment to the seminal vesicle, showed irregular

bands and whorls of cells.   Two principal types of cells were distinguishable: (*a*) polymorphous cells rich in protoplasm, with single vesicular nuclei or with multiple or large polymorphous nuclei; and (*b*) elongated and spindle-shaped cells possessing solid nuclei and closely associated with the connective tissue framework.   The arrangement was such that the cells rich in protoplasm lay within areas composed of spindle cells and connective tissue fibrils.   Blood vessels were fairly numerous and polymorphonuclear leucocytes richly infiltrated the tissue.   Under the medium power of the microscope the distinctions in the two types of cells became more evident. The large polymorphous cells made up usually by far the larger part of the tissue, and often were so fused together as to suggest syncitial formation.   In addition, there were present a number of multinucleated giant cells and cells with lobed gigantic nuclei distinct from the fused small cells.   Areas existed containing a preponderance of the spindle cells, but they never occurred strictly independently of the larger cells.   On the other hand, the spindle cells and fibrillar connective tissue tended to increase and diminish together. The collections of polynuclear leucocytes were larger in the degenerated foci, but they were not limited to the larger areas of degeneration.   The quantity of connective tissue in the tumor was likely to be underestimated from sections stained in hematoxylin and eosin (Plate I, Fig. 1).   The sections stained by the Mallory method showed the interstitial tissue to be much more abundant than was suspected, and the polymorphous cells to be surrounded by it.

Sections prepared from those parts of the original mass which were attached to the seminal vesicle exhibited besides what has been described still other structures.   In the first place, portions of the wall and epithelial membrane of normal appearance were included. Moreover, there occurred glandular structures which exhibited evidences of proliferation.   These later inclusions were of tubular nature, although the lumen of the tube was likely to be filled with projecting outgrowths of the walls.   These formations tended to be surrounded by structureless proper membranes, but sometimes the latter were imperfect, so that groups of epithelial cells passed from the tubule into the adjacent tissue.   Where this proliferation was going on the tubule had lost its regular and characteristic struc-

ture, and there developed small alveoli, consisting of polymorphous cells surrounded by spindle-cells and connective tissue, such as composed the mass of the tumor (Plate I, Fig. 2). In another part large tubules lay in hyalin connective tissue, which separated them from the usual tumor tissue, so that the formation of definite alveoli enclosing epithelial cells derived from the tubule was readily made out (Plate I, Fig. 3).

There was also discovered still another example of carcinomatous formation of striking and unmistakable character, which originated close to the epithelial wall of the vesicle. Sections through a part of the original growth, at a point showing to the naked eye small cavities and under the microscope cystic dilations of the inner glandular cavities of the vesicle, exhibited also irregular spaces lined with cubical epithelium and containing lumena in which there were desquamated cells and polynuclear leucocytes. These carcinomatous acini partially surrounded the cyst-like spaces which were included in the thickened fibrous wall of the vesicle supporting the tumor (Plate II, Fig. 4). These carcinomatous acini occurred also among the tumor cells proper, as well as in the fibrous stroma. In certain places spindle cells and fibrillated connective tissue encroached on the acini, compressing them and bringing about degeneration of the cubical cells, and the acini had in places been transformed into solid alveoli and thus made to resemble the cell groups shown in Fig. 1. Sections stained by Mallory's method showed the stroma to be abundant and to bear that relation to the acini and alveoli that is seen in a glandular organ.

We come now to a structure discovered within the mass, the explanation of which is impossible excepting on the basis of a developmental error. This structure consisted of a large space or tube, the walls of which were more or less corrugated and branching and covered with a layer several cells deep of high epithelium. Owing to the branching nature of the corrugations a certain number of elongated glandular tubular cavities appeared in the sections to be contained within the wall. The supporting connective tissue was richly infiltrated with small round cells. Adjacent to the large tube there lay a typical compound serous or mucous gland, and outside that structure a certain amount of smooth muscle existed (Plate II, Fig. 5).

The considerable variety of epithelial formations present in the tumor led us to examine microscopically the internal and external genital organs, and the seminal vesicles especially, of a number of white rats. Although there was some lack of agreement between the glandular structures contained in the tumor and those occurring normally in the vesicle, yet many of them appeared to be identical. A closer identification was, on the other hand, not possible with any other of the glandular parts of the internal genital organs. We failed entirely to identify the large tube with its associated serous or mucous gland with any structure which we discovered in the sections of the normal genital organs. It must, therefore, be concluded to have been an accidental and erroneous embryonic inclusion.

The foregoing description of the histological characters of the tumor indicates that it was not a simple but a complex formation, in which all the parts were not equal. This inequality was shown first in the body of the tumor, in which the polymorphous and spindle cells were united in a heterogeneous fashion, but it was shown even before in those parts in which the carcinomatous alveoli had developed (Plate III, Fig. 6). This absence of homogeneousness has an especial significance as regards the understanding of the interesting series of transformations through which the tumor passed in the course of transplantation through several thousand rats in the past three years.

It is not possible to establish the original tumor as a simple outgrowth from the seminal vesicle, as it was at first believed to be. As has been detailed, the more searching study of the original mass revealed epithelial structures differing materially from those of the seminal vesicles. The question, therefore, arises whether the tumor should not be regarded as a teratoma. If it is to be so regarded, then it follows that a part only of the epithelial structures contained within the tumor had been derived from the seminal vesicle, the other parts and perhaps the chief part of the epithelial inclusions having been derived from structures which under normal conditions would go to form adjacent epithelial organs.

It is also impossible to establish the precise origin of the cancerous transformation which occurred in parts of the tumor, and it cannot be stated with assurance that the transformation took place at many

points, rather than at one point.   The obvious interpretation to be put upon the appearances described in connection with those sections from which Figs. 2, 3 and 4 have been prepared is to the effect that they represent points at and structures with which this transformation is proceeding.   We rather tend to the opinion that the condition represented in Fig. 2 speaks for an active transformation of the glandular tubules into carcinoma, and yet the appearances are not wholly conclusive, since the reverse process, namely, that of cancerous invasion of the tubules from without, can not be excluded.

TRANSPLANTATION GENERATIONS.

In order that the vagaries of the tumor may be followed, and its properties established, some account of different transplantation generations will be given.   To make the account as brief as possible, merely characteristic examples will be chosen.   The first transplantation generation turned out luckily, since owing to inexperience only three rats were inoculated.   Small morsels cut with scissors were suspended in salt solution and injected into the peritoneal cavity and beneath the skin of three adult white rats on January 6, 1906.   The first of these was killed on February 8, and no tumor was found.   The two remaining rats developed intraperitoneal but no subcutaneous tumors.   Rat 2 was killed on March 19, at which time a nodule the size of a small bean was attached to the abdominal parietes.   Rat 3 died on June 26, nearly six months after inoculation.   There was no emaciation, but the skin over the abdomen was tense and the abdominal cavity contained a tumor the size of a walnut, attached to the abdominal wall and to the great omentum.   The tumor resembled the original in its gross appearances, although the central portion was necrotic but firm. Two smaller pea-sized nodules also occurred in the omentum; each kidney contained several metastases up to three millimeters in size; the lungs were studded with nodules ranging from a millet seed to a bean in size; a large nodule at the inferior border of the lungs united them to the mediastinum and diaphragm and extended into the pericardium; the inferior part of the pericardial sac was thickened into a cup-shaped depression for the apex of the heart;

the left ventricle was invaded from the pericardium and contained a nodule the size of a pea extending into the ventricle, while the visceral epicardium was generally thickened and white. On section the lungs were found to contain disseminated nodules, and the costal pleura contained several nodules.

It is interesting that Rat 3, which succumbed to the tumor developing within the peritoneal cavity, should have shown extensive metastases, while the original animal, which also showed an intraperitoneal growth, was not the subject of metastasis. Microscopical examination of the partially developed nodule from Rat 2 showed that the growth was proceeding from the periphery, while the center was already necrotic. The clearest picture illustrating the mode of growth was found at the point at which the abdominal muscle was being invaded. The cells of the outermost layer were small and round and resembled the usual small cell infiltration observed at the growing edge of malignant tumors in human beings. Mingled with them were oval epithelioid cells derived from the muscle and the interstitial connective tissue, and a moderate number of polynuclear leucocytes. Adjacent to this layer was a layer of capillary vessels separated from one another by oval and elongated cells and a fine fibrillar tissue. This one, which was very cellular, passed over into the fibrous layer, which was more cellular at the periphery than at the center. This fibrous strand was not strictly homogeneous, but included islands of large cells corresponding with the polymorphous cells, rich in protoplasm, of the original tumor. In the central fibrous tissue small islands of these cells were undergoing necrosis. There were present a certain number of multinucleated masses of protoplasm, constituting giant cells, or resembling syncytium. Sections stained by Mallory's method brought out the fact that almost everywhere in the tumor the connective tissue was so arranged as to form definite spaces enclosing oval and polyhedral cells with large vesicular nuclei. The tumor was, therefore, not a homogeneous structure anywhere, but was made up of two elements, connective tissue and cells independent of but enclosed in it.

The microscopical appearance of the tumor nodules in Rat 3 will be described. The large omental nodule showed a cortex merely of

living tissue, and center consisting of hyalin and fibrous tissue. The most peripheral zone was the most cellular, but also contained much fibrous tissue. The cells consisted chiefly of the large polymorphous cells, with vesicular nuclei occurring singly and in groups. The center, which was hyalin and fibrous, still showed areas of the polymorphous cells. These cells and the cells forming the capillaries and the stroma were quite distinct. The smaller omental nodules represented developments within the adipose tissue. The most recent invasion was at the periphery, which consisted of areas of the polymorphous cells embedded in hyalin connective tissue. These nodules were inactive and the centers, already hyalin, enclosed atrophying cells of the large type.

The lung nodules might or might not involve the pleura. When the intrapulmonary nodules were small they surrounded the medium-sized blood vessels and consisted of the large cells occupying the perivascular lymph spaces, from which they spread into the alveoli. Apparently the alveolar epithelium was lost and the spaces were entirely filled from the larger tumor cells. ·Unless the pleura was invaded there was no increase in the connective tissue, but invasion of the pleura at once led to a new growth of connective tissue, which together with the tumor alveoli quickly underwent hyalin and other forms of necrosis. However, in the hyalin tissue a certain number of columns of the large tumor cells persisted. Usually the lower border of the tumor in the pulmonary parenchyma preserved its integrity. In addition to the usual cells, multinucleated giant cells occurred in the pulmonary nodules. Sections carried through the large nodule at the hilum of the lung from which the pericardium was involved showed appearances similar to those of the large intraperitoneal mass, but in addition there were present a remarkable number of mono- and multinucleated giant cells, some of which were of the megacaryocytic type (Plate III, Fig. 7). These cells often included mononuclear and polynuclear leucocytes. Some of the massive giant cells included scores of smaller cells, probably leucocytes, in process of fragmentation. The giant cells were irregularly distributed. The great blood vessels were surrounded by tumor, but were patent. An intermediate stage was present in this part of the lungs, between the

early condition in which the alveoli were occupied by tumor cells, and the later one, in which the tumor had become hyalin and fibrous. This intermediate stage was characterized by a thickening, through spindle cells and fibrillated tissue, of the air vesicles, which contained mononuclear cells of the type of the tumor cells, suggesting when viewed under the low power of the microscope an alveolated tumor of the epithelial type. Mitotic figures in the large cells were frequent. The nodules in the parietal pleura were invading the intercostal muscles on the one side and projecting into the pleural cavity on the other. They lacked the organoid characters described and were composed of spindle cells coursing longitudinally and transversely, and of small round cells next to the muscle, and were invaded with polynuclear leucocytes.

The masses in the kidneys, the largest of which measured six by four millimeters, developed in the cortex. These nodules, some of which were wedge-shaped, were produced by an invasion of the tubules of the kidney and of the perivascular connective tissue with the large polyform tumor cells, which had taken the place of the proper epithelium of the tubules. Among them a few multinucleated giant cells were present. The glomeruli persisted much longer than the tubular structures and the intertubular connective tissue was increased by a layer of fine connective tissue fibrils and spindle cells. Polymorphonuclear leucocytes were distributed throughout, and accumulated most numerously in minute foci of necrosis. When the nodules projected beyond the capsule they consisted chiefly of connective tissue containing a certain number of large cells occupying definite spaces suggestive of lymphatics. When these lines of cells were longitudinally distributed, they sometimes extended across the field of the microscope, and when they were cut obliquely or transversely, they resembled alveoli.

The nodule in the ventricular wall consisted of a solid tumor composed of a fibrillated stroma, spindle cells and capillary vessels, in which the large tumor cells were embedded in an irregular manner. There was absence of alveolation, and the tumor cells proper formed small groups or occurred singly, and were closely surrounded by the stroma. Giant cells were also numerous. At the extending edge of the nodule a zone of small round cells

appeared, from which capillaries penetrated the muscle fibers. These fibers were separated and compressed by spindle and small round cells, a formation disclosing the invasion of the tumor cells proper, which passed between the muscle fibers in parallel lines. The cells that appeared to be the most recent consisted of multinucleated masses of protoplasm. What was taken to be the coronary sinus had been invaded by the large cells, which were unprovided with a stroma. At one point the lumen was bridged by the tumor cells and at other places a fibrinous thrombus had developed, into which the tumor cells were pushing (Plate IV, Fig. 8). The general layer of epithelium was thickened and infiltrated with a mixture of the large tumor cells and spindle cells, and was excessively vascularized. It is patent that this transplantable tumor possessed a considerable degree of invasive power, while it exhibited, on the other hand, relatively slow growth. Death was caused apparently not by interfering with the nutrition of the animal, but rather by mechanical causes, chiefly probably connected with the extensive lesions of the lungs.

The structure of the tumor, as revealed by the first transplantation nodules, was sufficiently varied to make classification very difficult. The tumor was not, apparently, homogeneous in nature, as are the usual sarcomata, but rather of heterogeneous composition, such as is found among the epithelial tumors. The point should be emphasized that all the metastases discovered were such as could best be produced through the general blood current. There was no evidence of involvement of the lymphatic glands anywhere, but that the lymphatic vessels of the viscera could be invaded was indicated by the conditions observed in the lungs and the capsule of the kidney. Direct infiltration of the serous membranes through implantation occurred in the peritoneal cavity and in the epicardium.

In the course of the different generations considerable variations in histological structure and metastatic properties, which it is not necessary to describe in detail, were presented by the tumor. On the other hand, it is desirable to present the important facts regarding the peculiar behavior of the tumor. The generations represent a continuous series, while different strains within a generation, expressed by letters of the alphabet, do not represent a uniform

series, but indicate merely the different sets of inoculations, usually from different nodules, within a generation. A given generation will, therefore, contain one or more strains according to the circumstances governing the transplantations. These facts are of some importance, in view of the circumstance that the tumors were often histologically dissimilar within a given generation, as will be shown.

ABSTRACT OF PROTOCOLS OF THE RATS WITH TUMORS.

In this section there will be presented a selected series of protocols, briefly abstracted in order to bring out the varying and changeable properties of the tumor.

Of the second generation, strain *a,* Rat 7 survived four months and presented a large ulcerating subcutaneous mass and pulmonary and diaphragmatic metastases. Rat 8, inoculated at the same time as 7, developed a subcutaneous tumor which ulcerated later, as a result of which it survived a month longer and showed only pulmonary metastases. Rat 6, of the same series, survived two weeks longer than Rat 7, and showed, besides the subcutaneous nodule, kidney metastases.

Rat 64 of the fourth generation (*a*) survived five and one-half months. The tumor which first developed was partially excised for inoculation purposes, but it recurred and eventually caused death. It invaded the muscles and peritoneum and projected into the abdominal cavity posteriorly. Metastases were present in the sternum, cartilage of the ribs, diaphragm, left thoracic wall to a marked degree, right thoracic wall to a small degree, the anterior mediastinum, the lungs and left kidney. The slower progress of the tumor was the result of the excision, since other rats of this series not operated upon succumbed within three to four months of the inoculation. Other rats showed lung metastases and one, Rat 66, a metastasis in the auricular appendage. Rat 47 is of special interest in this connection. It was inoculated into the peritoneal cavity with a single fragment of tumor and survived about four months. The main tumor, which developed from the fragment, was situated in the omentum, but secondary nodules were present elsewhere in the abdominal cavity (Plate XIII, Photograph 1). One of these, measuring 1 by 2 centimeters, was attached to the ensiform cartilage, which proved to be a point of predilection for the development of intra-peritoneal nodules. A second point of predilection, as it afterwards proved to be, was the space between the liver and the diaphragm, next to the cardiac extremity of the stomach. In this instance the stomach had been invaded by the tumor, which had passed to the mucous membrane and led to a partial digestion of the membrane and production of a cup-shaped depression. A single pin-point metastasis existed in the lungs. Rat 46 was also inoculated into the peritoneal cavity. It survived about three months. The original fragment developed in the pelvis to a tumor measuring 3 by 2 by 1½ centimeters, which had invaded the muscle of the abdominal wall. A series of

secondary nodules developed within the omentum and extended upwards to the transverse colon and in the gastro-hepatic omentum, reaching the diaphragm and binding it to the lesser curvature of the stomach. The cardia of the stomach was invaded over its entire extent at the diaphragm by the tumor, which passed into the cavity and replaced the mucous membrane, which became altered to form a cup-shaped depression (Plate XIV, Photograph 2). Separate small nodules appeared over the peritoneal covering of the stomach, and the lungs showed a number of small metastases. Rat 61 survived a subcutaneous inoculation about three months. The superficial tumor which developed at the lower border of the thoracic wall measured 3 by 4 centimeters. It penetrated the muscles and projected into the abdominal cavity, pushing the retro-peritoneal tissue before it. As a result the right kidney and adrenal gland were forced forwards, the kidney was extensively invaded with tumor, the right adrenal gland was converted into tumor, and a mass of tumor extended in the gastro-hepatic omentum towards the lesser curvature of the stomach, over which it spread and into which it penetrated. An elevated boss-like nodule appeared in the mucous membrane and was connected with the mass in the wall; while on the opposite (left) side a larger ulcerated area existed, probably representing an implantation tumor upon the mucous membrane through contact (Plate V, Fig. 9). Rat 67 survived five months and was inoculated on two separate occasions. The first tumor developed in the subcutaneous tissues and ulcerated. The second fragment was introduced into the lumbar muscles, developed there and extended upward, invading the sternum and ribs. The left pleural cavity was quite filled with a mass which compressed the left lung entirely. At the same time it grew downwards, displacing the diaphragm inferiorly, and upwards to the apex of the thoracic cavity, pushing through the ribs and intercostal muscle and came to lie beneath the superficial fascia. The pericardium and mediastinum were overgrown and the right pleura, which was invaded, led to the binding of the lung to the ribs. Rat 51 survived an intra-abdominal inoculation about four months. A main mass 5 by 5½ centimeters developed and secondary small nodules were produced. The mesentery was invaded, thickened, and caused to retract, so as to bring the small intestine into a close small coil. The right kidney was surrounded by a mantel of tumor; the diaphragm, and from it the pleuræ, were invaded, and ascites existed. The ascitic fluid measured 25 centimeters and contained masses of tumor cells.

As will be observed, in the course of the fourth generation, in which a considerable number of rats was inoculated, a considerable variety of effects were produced. What is particularly striking is the invasiveness exhibited by the tumor and its tendency to develop frequently in the region of the diaphragm, adjacent to the right lobe of the liver and the stomach, and to avoid invading the former and choosing regularly to invade the latter. In no instance during this generation was a lymphatic gland metastasis observed.

In the fifth generation some interesting examples were observed. Rat 325 (g) was inoculated into the muscles of the back and survived 2½ months. The tumor which developed measured 2 by 2 centimeters. It invaded the ribs and spinal column in the dorsal region, produced paraplegia, and, projecting into the thoracic and abdominal cavities, displaced forward the liver and kidney. The

lungs and mediastinum contained nodules. Rat 327 was killed after three months, at which time there existed besides the subcutaneous nodule and the lung metastases a mass in the region of the axillary gland which measured 0.4 by 0.4 centimeter. It should be remarked that this was the first example of apparently a lymphatic gland metastasis. Rat 276 survived 2½ months. A sub-cutaneous nodule of the right side had penetrated the abdominal cavity, pushed the kidney and adrenal gland forward, displaced the spinal column to the right, and given rise to a secondary nodule compressing the ureter, which above the nodule was dilated to the size of a goose quill, and was associated with a hydro-nephrosis in which the kidney was enlarged to double its normal size. There were other nodules in the omentum and serous covering of the spleen and liver, in the mediastinum, and between the heart and the lungs. Rat 196 (*d*) sur-vived three months. The original ulcerated subcutaneous nodule involved the skin and abdominal muscles and penetrated the lower ribs into the abdominal cavity. In the subcutaneous tissue above the main tumor was a separate smaller nodule connected with the original tumor by a white cord. The second nodule was in turn connected by a similar white cord with a third nodule, situated in the axillary space and representing probably the much enlarged axillary glands. The white cords were taken to be lymphatic vessels invaded by tumor. Rat 197 showed, as the result of a second implantation, tumor invasion of the inguinal lymphatic gland on the side of the inoculation, and other metastatic nodules. Rat 230 exhibited invasion of the ribs, penetration of the thoracic cavity and the spinal canal, associated with paraplegia. The infra-clavicular lymphatic glands on both sides were invaded. The superior lobes of the lungs were studded with nodules, the caudal lobe was the seat of a diffuse infiltration, a lymphatic gland above the diaphragm was enlarged by tumor to the size of a pea, and the kidney contained a nodule 2½ centimeters in size. Rat 231 showed a similar condition to the last, and in addition, a retro-peritoneal and bronchial gland, as well as the left axillary gland, contained metastases (Plate XIV, Photograph 3; Plate XV, Photograph 4). Rat 93 (*a*) survived 3½ months. A secondary graft developing subcutaneously involved the axillary gland, which became enlarged to a size of 1 by 2 centimeters and from which a growth sur-rounded the clavicle, penetrated into the pleural cavity, and encircled the great vessels of the heart. The lungs presented nodules, but the heart did not. Rat 88 survived an intra-muscular inoculation two months. The main tumor forced its way into the thorax on the left side, pressed against the vertebral column, which was displaced anteriorly and to the right. The ribs corresponding to the main portion of the tumor had disappeared, the lower half of the left pleural cavity was obliterated by the mass, which invaded the diaphragm, penetrated into the abdominal cavity, grew into the cardia of the stomach, the superior pole of the kidney and the adjacent adrenal gland and involved the lymphatic glands at the superior surface of the liver. Rat 418 (*h*) developed a subcu-taneous tumor which ulcerated and led to axillary and inguinal lymphatic gland metastases. In the inguinal region a chain of enlarged glands or nodules extended from the margin of the tumor to the angle of the leg. Pulmonary and mediastinal metastases occurred. Rat 424 (*i*) was inoculated intra-peri-toneally with ascitic fluid from Rat 51. Multiple intra-abdominal nodules de-

veloped in the omentum at the ensiform cartilage and between the spleen and the left kidney. A chain of nodules extended from the pelvis to the stomach, the wall of which at the expanded esophageal part was invaded and the mucosa ulcerated (Plate XV, Photograph 5). Rat 425 developed an intra-abdominal tumor growing about the stomach, spleen, left kidney and adrenal gland. A nodule had passed completely through the spleen, and the left adrenal gland was lost in the growth. Rat 426 exhibited two large and many small intra-abdominal tumors. The inoculation was made with ascitic fluid in the manner of Rat 424. The left kidney was replaced by tumor, except for a thin shell of renal tissue surrounding a large cyst.

In the fifth generation, therefore, the tumor began to produce lymphatic gland metastases, and at the same time continued to be deeply invasive of tissues in its locality, to be disseminated by implantation, and to produce, as before, abundant distant metastases. In the succeeding generations the properties of the tumor as now developed did not undergo any striking alteration. A few notes may be made, therefore, with reference to certain points which appeared from time to time. For example, it was noted in the seventh generation that seven additional examples of gastric invasion occurred. The invasion always took place at the dilated esophageal extremity, and from the tumor growing between the liver and the diaphragm. The kidneys were not infrequently the seat of metastasis. Sometimes one kidney would be quite converted into tumor (Rat 597, seventh generation), another example of compression of the ureter, succeeded by hydronephrosis, was noted in the tenth generation. The pulmonary metastases were much the most common of all. As a rule, when lung nodules existed, the mediastinum was invaded and usually the parietal pleura and the intercostal muscles became infected. Sometimes an entire lung was invaded from the hilum (Rat 1775, tenth generation), and compression of a branch of the pulmonary artery by tumor gave rise to infarction of a lobe of the lung (Rat 401, seventh generation). The myocardium was invaded in several additional instances. A mass extended from the lungs into the wall of the heart (Rat 553, sixth generation). A metastatic nodule appeared in the left ventricle (Rat 401, seventh generation) and implantation nodules occurred in both auricles and in the left ventricle (Rat 2392, ninth generation) The lymphatic metastases continued to appear. They were usually in the axillary and inguinal glands, and arose from subcutaneous inoculation. However, other superficial and even the deeper glands were sometimes involved. The retro-peritoneal glands (Photographs 3 and 4) were several times involved, and once a chain of these glands was affected, along with the axillary gland (Rat 1648, eleventh generation). A pelvic lymphatic gland was once involved, but afterwards suppurated (Rat 3035, fourteenth generation). The bronchial lymphatic glands, together with the axillary glands (Rat 1860, tenth generation) and once the bronchial, trachæal and clavicular glands were invaded jointly (Rat 1617, eleventh generation). The inguinal, axillary and mesenteric glands were once jointly invaded (Rat 2048, tenth generation). In one instance, a tumor nodule pressing upon the common bile duct produced jaundice (Rat 1338, ninth generation). In two instances the wall of the thoracic duct showed small nodules (Rats 1753, tenth, and Rat 2273, eleventh generation).

So much has been stated of the tendency of the large tumor masses to set up metastases that it remains to be added that the tumor could grow to a considerable size, produce marked local destruction of tissue, without at the same time yielding any discoverable metastases.

The object of presenting such a considerable number of protocols is to bring out the fact of the malignancy of the tumor under discussion, and the great variety of effects which it produced according to the circumstances of its development. The tumor was found to recur after what appeared to be complete surgical extirpation, to grow through the skin, muscle, fascia and bone, and to penetrate into the circulation, thus setting up secondary or metastatic foci at a distance. It should be noted that until the fifth generation there had been no instance of lymphatic gland metastasis, and during that generation and in subsequent generations this form of local metastasis became common. As will be observed a little later, the acquisition of the property enabling the tumor to invade the lymphatics was associated with a significant histological alteration in its structure. The growth within the abdominal cavity tended to become multiple, even when they arose from a single inoculated fragment, a result to be expected in view of the conditions in the peritoneal cavity which favor the secondary implantation of tumor cells. It is, however, noteworthy that these implantations were so common between the diaphragm and the stomach, which structures tended themselves always to be invaded. Of all the intra-abdominal organs, the stomach was most frequently penetrated by the tumor, which tended to appear in the mucous membrane and to set up ulceration quite as tumors do in man which originate in the mucous membrane. It is further remarkable that the gastric nodules always developed in the esophageal segment of the stomach, and that the mucosa was subject to infection directly through contact with tumor on the opposite side of the viscus. It is worth considering, therefore, whether the tumor can be implanted on the normal mucosa. We have abundant evidence to show that rats cannot be infected by feeding upon the tumor. It was repeatedly observed that when fragments of the tumor were inoculated into the muscles they tended to develop towards the interior, and when implanted beneath the skin, towards the surface of the body.

Cartilage and bone offered no impediment to the growth of the tumor. The deposit by preference of the tumor emboli in certain organs, such as the lungs and kidneys, is a distinctive feature of the tumor, as is the tendency of the lung nodules to lead to infection of the mediastinal tissues and the walls of the thorax, the latter apparently through contact. Metastases were never developed in any of the internal organs or lymphatic glands except through blood infections, although they might be produced by direct extension from neighboring parts. It is probable that the nodules in the heart muscle were all the result of ingrowth from the lungs, and it is remarkable that with the frequency of the pulmonary metastases the bronchial lymphatic glands were so rarely affected. The superficial lymphatic glands were much oftener affected than the deep ones. Small and medium-sized and ulcerated tumors, in which only a thin membranous remnant of tissue remained, might produce many metastases, and large, well developed tumors might produce no metastases. It should be noted that in one instance a nodule compressed the common bile duct and led to jaundice; in two instances small nodules were observed in the thoracic duct.

HISTOLOGY OF THE LATER TRANSPLANTATIONS.

It is not our intention, in considering the histology of the transplantation tumors, to describe exhaustively the successive generations. The effort will be made, however, to present the facts of the histology in such a manner as to bring out the significant and fundamental histological changes through which the tumor has passed, and with which have been correlated certain properties of metastasis, and to make clear minor fluctuations in structure that are being slowly eliminated. In another section there will be discussed the manner of proliferation of the tumor fragments, so that this point can be passed over now.

The second and third generations of the tumor showed no remarkable variations of the structures already described (see page 19). A variation was found according as the tumor was proliferating or had come to a temporary standstill. According to one or the other, two quite independent types could be distinguished:

(1) a proliferating tissue, producing stroma and columns of epithelial-like cells (Plate V, Fig. 10; Plate VI, Fig. 11; low and high power); (2) a stationary tissue composed of capillaries and spindle cells and few epithelioid cells (Plate VI, Fig. 12). The two types were grossly so different that had it not been for their common origin and certain resemblances between the epithelial cells they could not be considered as related. What should be perhaps emphasized is the fact that in retrogressing hyalin fibroid tissue associated with the stationary nodules the most persistent cells were certain epithelioid elements occupying compressed spaces in the hyalin mass (Plate VII, Fig. 13).

In the fourth generation (*a*) the appearances were various. An omental nodule in Rat 45 was composed wholly of elongated, closely packed cells, without arrangement into alveoli, and the nodule was quite solid except where islands of adipose tissue remained free of tumor. In Rat 49 the pancreas had been invaded and the tumor had produced obliteration of the splenic vessels, causing necrosis of the spleen. In respect to histology, this tumor was imperfectly alveolated or fibrous. The growth in the pancreas was highly fibrous and included isolated tubules resembling ducts, lined with superficial epithelium, doubtless the remains of the excretory ducts of the gland. The walls of the obliterated splenic artery and vein, which contained a hyalin and leucocytic thrombus, showed inflammation and the passage of the large tumor cells from without into the lumina (Plate VII, Fig. 14). In other sections the splenic artery showed an obliterating endarteritis of high degree, the spleen, a wedge-shaped growth of tumor about the vessels, and the liver, a superficial invasion attended with necrosis of adjacent hepatic lobules. In Rat 50 the small intrapulmonary nodules consisted of the air vesicles, in which the normal lining had been substituted by the large tumor cells. The pleural nodules were larger and the epithelial masses within them exhibited an acinous arrangement. When the pleural mass projected beyond the membrane the number of living tumor cells was small and it was composed chiefly of hyalin fibrous tissue, or a homogeneous tissue into which the large pale degenerating tumor cells with large hypochromatic nuclei were being fused. The portions of the nodule immediately within the

pleural membrane and extending superficially into the lung were composed of large, irregular alveoli containing epithelioid cells, often enclosing clefts suggesting lumena. Some of these alveoli had become dilated into small cysts enclosing cell detritus. The large epithelioid cells were dividing rapidly by mitosis, while no mitoses were seen in the stroma. The tumor, which was extending into the parietal pleura and the intercostal muscles, preserved its alveolar character, although the amount of stroma being produced was highly excessive. The manner of gastric invasion was characteristic (Plate VIII, Fig. 15). In the first place the growth was limited to the dilated esophageal segment. The section viewed from the inner surface of the organ showed a cup-shaped depression over the central and part of the peripheral portion of the growth, which rested on necrotic tissue devoid of nuclei. The mucous membrane was persistent at each side of this crater. The main or central mass of tumor was highly fibrous and resembled in structure a cicatrizing, scirrhous carcinoma in man. This dense fibrous portion still showed a small number relatively of compressed areas of cells, originally the large epithelioid cell. A sharp but iregular line of demarcation composed of fragmented leucocytes and bacteria marked the limits of living and dead tissue. The extreme edges of the tumified mass showed more tumor tissue proper, less fibrous tissue, and some remains of the smooth muscular wall of the organ. Where the mucous membrane was still preserved, the epithelioid tumor cells occupied spaces between the connective tissue fibrils that formed the submucosa and penetrated the papillary layer. As soon as the papillary layer was invaded, two kinds of pathological changes took place in the mucosa. The first affected the inner or superficial face of the epithelium; the continuity of the horny layer was disturbed and collections of fluid in vesicles formed. These vesicles penetrated only a short distance into the substance of the interpapillary epithelial processes, but they projected for a much greater distance into the cavity of the organ. The contents of the vesicles were homogeneous and contained leucocytes and fragments of keratinized epithelium. The epithelial layer below was much disturbed. The second change consisted of a deepening of the interpapillary processes, which also became narrowed and penetrated

more deeply into the tumor-infiltrated sub-mucosa.   Moreover, they had divided irregularly into digits.   The epithelial cells composing them had become paler and richer in protoplasm and were actively dividing by mitosis.   As the edge of the crater was approached the dilatation was most marked and the horny layer imperfectly formed and rapidly disintegrating.   In other words, the epithelial layer of the esophagus, at the point of beginning invasion of the tumor, was caused to·undergo atypical proliferation. Rat 61, which also showed invasion of the gastric wall, was interesting in virtue of the fact that it brought out the difference in vulnerability of the esophageal and glandular portions of the organ.   The growth was mainly confined in the muscular wall and the peritoneal coat, both of which were lost in the mass.   The mucous membrane of the esophageal segment was invaded and ulcerated, the invasion ceasing a fraction of a millimeter before reaching the glandular segment.   At the point of cessation in the esophageal territory the muscularis mucosæ formed the boundary line, although the tumor cells had penetrated between it and the muscular tunic, which had now become widely separated.   In the normal organ the demarcation between the two anatomical parts—the esophageal and glandular portions of the organ—is distinct and indicated by a finger-like projection of sub-mucous connective tissue and smooth muscle fibers, as well as by the change in epithelium.   The squamous epithelium ends on the glandular side of the projection, although the esophageal layer has lost its papillary character and become thinner before it disappears.   The limits of the tumor were shown also on the outer surface of the stomach, since the peritoneal and muscular tunics, corresponding with the glandular part of the organ, showed no invasion, and the omental adhesions were limited wholly to the mass in the esophageal region.

The kidney nodules were developed chiefly in the cortex, the proper struture of which was replaced by the tumor cells.   The latter invaded the tubules and took the place of the normal epithelium, from which they were distinguished by their greater density of protoplasm and greater richness in chromatin of the nuclei.   The tumor cells formed a lining similar to the old, but sometimes a double one.   The inter-tubular tissue was increased; it had become homo-

geneous and rarely showed the presence of the large tumor cells, but the compression and obliteration of the tubules, the epithelium of which was replaced by tumor cells, through the new connective tissue growth, sometimes produced appearances resembling an extra-tubular invasion. A certain amount of glomerular obliteration had taken place through hyalin thrombosis. The central mass of the tumor, except small islands immediately surrounding blood vessels, was entirely necrotic, and the walls of the vessels had been invaded and the endothelium had sometimes been displaced inwards by the tumor cells, although the lumena were still patent.

The subcutaneous tumors became attached very late to the skin, because of the great resistance to invasion exercised by the corium (Fig. 10). Examples show that the growth in the corium tends not to be continuous, but to be initiated apparently by large epithelioid cells, which are carried to a distance apparently in the tissue spaces, where they proliferate and start the production of a young stroma. The stroma of the tumor proper is sometimes more subject to degeneration than the epithelioid cells. An example of this condition was presented by Rat 53, in which the stroma was necrotic and freely invaded with leucocytes undergoing fragmentation, while the epithelial strands retained their form and appearance, except for the fact that the protoplasm was more strongly basophilic than usual. The numerous blood vessels were dilated and many contained hyalin or agglutinative red corpuscular thrombi.

From the description of the foregoing examples it would appear that the tumor had acquired in the fourth generation the unmistakable characters of a carcinoma, yet in point of fact the carcinomatous type was not really established in this generation, since the tendency for the epithelioid cells to be in close relationship with the stroma had not been entirely lost. Rat 57 illustrates this point.

The tumor when about one centimeter in diameter was in part excised for purposes of transplantation. The cortical zone, which was employed, showed under the microscope elongated bundles of highly cellular connective tissue enclosing small groups of epithelioid cells. The young tissue consisted of spindle cells which were particularly massed at the periphery, while the central part was more fibrous or even hyalin. While the epithelioid and connective tissue elements

were intermingled at the periphery, the former were well defined in the fibrous stroma. At the death of the rat, a month later, the recurrent nodule proved to be highly fibrous, and showed epithelioid cells in rows and islands in process of compression and atrophy.

We shall now consider a few examples of the fifth generation, which, as will be recalled, was characterized especially by the appearance for the first time of lymphatic gland metastases. It was in this generation also that several examples of compression of the spinal cord occurred. An example of the latter condition will now be described.

Rat 87 (*a*) had been inoculated into the muscles of the back, and the growth had extended toward and invaded the spinal column. Viewed under the microscope the growth was composed in its preserved parts of the large epithelioid cells forming solid alveoli which had penetrated all the soft parts, substituted itself for them and surrounded the vertebral column and ribs (Plate VIII, Fig. 16). The spinal nerves at their emergence from the canal were invaded. The large tumor cells insinuated themselves between the muscle fibers, which were caused to atrophy, and between the nerve sheaths. The bony parts of the vertebræ were not destroyed, excepting the spinous processes, in which no bone remained. The tumor passed to the spinal canal and reached the dura mater, but the spinal cord was not compressed. It is probable that the paralysis was produced through involvement of the spinal nerves roots. Rat 198 (*d*) showed multiple pulmonary nodules, in which the tubular or acinous arrangement of the epithelial cells was well marked. The nodules were often as large as a pea and replaced the pulmonary tissue. The stroma was finely fibrillated and did not conform to the original architecture of the lung. The pleural nodules were particularly interesting. While they possessed a denser and more abundant stroma, they were covered on the free surface with a layer of columnar epithelioid cells proliferating rapidly by mitosis, which on dipping down into clefts and depressions of the tissue gave rise to typical acinous tubules, as viewed in sections. A subcutaneous nodule in the same animal showed similar epithelioid cells proliferating rapidly and forming linear solid strands and not acinous tubules. There was also in this animal an ingrowth of tumor into the vertebral column, which invaded the dura mater but did not infiltrate the spinal cord. The roots of the spinal nerves and the inter-vertebral ganglia were surrounded. This tumor mass showed interesting variations of type. The older and more superficial parts were in a state of coagulative necrosis. Immediately next to them was a mass of solid anastomosing strands of cells pushing into and replacing the muscle fibers, which showed all stages of atrophy. The stroma here developed was highly fibrous. The large tumor cells surrounding the spinal nerves developed in the perineurium and produced a heavy collar of cells about the nerves, so that the main trunks of the nerves were completely degenerated. The type of tumor cells developed within the fibrous stroma and leading into the spinal canal was smaller than elsewhere; the nuclei were darker and the arrange-

ment was radial about the irregularly anastomosing spaces containing the cell masses, so that they were made to resemble in a remarkable way the normal cells lining the narrow spaces. Where the cells could grow more freely in the muscles and fascia beside the vertebral column, the alveoli became more regularly rounded or branching. Here and there a central excavation had taken place among the cells, producing a kind of acinous arrangement, to which the term pseudo-tubular might be applied (Plate IX, Fig. 17). At the outer termination of the muscles, where the growth might be considered as having been free to extend, the cells were large and pale, and were rapidly multiplying by mitosis; they were disposed into columns and made to resemble the form of growth described in the pleural metastases. Rat 411 (h) showed some interesting features. There was an extensive tumor growth at the root of the lung involving the great vessels of certain lobes. The vessels were thrombosed and the corresponding lobes of the lungs were necrotic. The tumor passed at times into the interior of the vessels, so that epithelioid cells came to lie between the inner wall and the thrombus in a narrow slit or space, giving rise to tubules containing large multi-nucleated cells. Elsewhere in the lungs there were numerous tumor nodules developing about the bronchi and blood vessels, which penetrated their walls but did not enter the lumena. The tumor cells in these cases might come to lie just between the endothelium of the vessels and the epithelium of the bronchi. In these the alveolar formation was solid. The kidney nodule showed replacement of the parenchyma of the organ by the tumor, the epithelioid cells of which occupied the pre-formed tubules, but the stroma was newly formed.

We shall consider now the examples of lymphatic gland involvement. As stated, the tendency of the tumor at this time was to exhibit the structure of carcinoma. The particular type presented was that of simple carcinoma, in which the alveoli showed great diversity of form, but contained solid masses of epithelioid cells. In the course of the fifth generation, however, this simple form began to show modifications, tending to the acinous arrangement, and although the two types, the solid and acinous, of alveoli were fluctuant, yet the former became more and more common in this and subsequent generations, until it became dominant. We shall pass to the seventh generation before taking up the consideration of the nature of the lymphatic gland metastases.

Rat 404 (d) showed, besides the growth in the regional lymphatic glands, metastases in the lungs. The subcutaneous nodule had ulcerated and left a mere capsule of fibrous tissue containing strands of epithelioid cells. It showed no acini. The lungs showed both disseminated miliary and larger nodules, and one lung was quite completely converted into tumor. The main blood vessels leading to this lung were surrounded by tumor and thrombosed, the thrombi being in process of organization. The bronchi had been either surrounded or

invaded by tumor. The pleura also contained nodules. The lungs were partly bound to the chest wall by the tumor growth, and the areolar tissue under the heart and lungs was invaded, but the growth, which extended to the lymph glands in this areolar tissue, had not invaded them. The type of the tumor was the simple alveolar. On the other hand, the lymphatic glands in the region of the superficial ulcerated mass had been invaded, some of them almost entirely replaced by the tumor. The epithelioid cells were pale, some of them presented hypochromatic nuclei, and they formed solid alveoli (Plate IX, Fig. 18). The succeeding animals frequently showed a similar replacement of the regional lymphatic glands, either with solid alveoli or a branching type of the tumor, and in rare instances the tumor passed through the lymphatic glands into the periglandular adipose tissue.

Rat 729 (*d*) of the eighth generation showed the manner in which the lymphatic glands sometimes become infected. The local tumor, of fibrous nature and containing only a few of the epithelioid cells in small groups, approached the gland and became fused with the capsule, after which blunt papillary-like processes, consisting of large epithelioid cells, pushed into the superficial lymphatic tissues, which was the starting point of the invasion.

We return for a moment to Rat 401 (*d*) of the seventh generation, because of a nodule contained within the auricle of the heart and penetrating into the cavity of the auricular appendage. The muscle cells in the region of the tumor were either undergoing atrophy or had entirely disappeared. The tumor itself was an alveolar growth of solid type, possessing a rich fibrous stroma. The ingrowth into the muscle of the heart was accomplished through buds of multinucleated protoplasm, which, enlarging, produced the strands and oval alveoli. The cavity of the appendage contained a fibrinous clot surrounded by hyalin muscle fibres, into which the tumor had penetrated and produced a tubular formation.

At about this time the tumor showed now and then a much softer structure than it had previously done, which can best be illustrated by one or two examples. Rat 808 (*d*) of the eighth generation possessed a tumor the size and shape of an almond, that was composed of cell masses made up of large pale epithelioid cells arranged either in solid alveoli or in tubules, showing lumina. The cortical portion was made up of living cells, while the central part, except for the tissue immediately about the blood vessels, was the seat of a soft necrosis, quite different from the hyalin necrosis usually observed. In this necrosis, which resembled an anæmic infarction, the cell forms were still present, but distorted and discolored and

mingled with fragmented nuclei. This particular form of the tumor became progressively more common, and led to the typical adenoma that constituted the final form in which the tumor occurred. In different examples there was some difference in arrangement of epithelioid cells and in relation of cells and stroma, but the properties were generally preserved. What was particularly impressive in this type of the tumor were the frequent large mitoses in the epithelioid cells.

Rat 743 (*d*) was another example of this type of tumor, which was even more perfectly adenomatous. The mass was several centimeters long, oval in shape, and possessed a fibrous capsule. The cortical layer, still living, was about two millimeters in thickness, while the central part was necrotic and soft, but still showed small islands of living tissue about the capillary and larger blood vessels. There was little or no stroma, and the bulk of the growth consisted of epithelioid cells, proliferating very rapidly and, therefore, showing numerous mitoses. The individual cells were ill-defined, but multinucleated bands and rows of cells had been produced. At the outermost edge of the tumor the epithelioid cells were narrow and often linear, but further inward they were wider, formed oblong masses and frequently showed a peripheral circle about a lumen. There was much curving and bending of the epithelioid cells, so that acini of a variety of shapes were produced. In the islands of tissue left preserved about the vessels in the interior, a tubular, acinous arrangement nearly always was present (Plate X, Fig. 19).

To illustrate the fluctuations which were still going on at this period, Rat 1280 (*i*) of the ninth generation will be used as an example. Besides a nodule in a lymphatic gland, which perforated the capsule and grew into the surrounding areolar tissue, the original nodule was composed of simple alveoli containing pale cells and showing numerous mitoses. The amount of degeneration was not excessive, and no tubules or acini were produced. Two other points may be mentioned: one of the large superficial nerves was surrounded (Plate X, Fig. 20), and a number of mammary gland ducts were included in the growing tumor. This latter condition was by no means uncommon when the implantations were made in the region of the mammary glands of female rats. Frequently

these ducts persisted in the tumor and showed little change. At other times their proper membranes were destroyed and the ducts themselves disintegrated. Rat 2402 (*o*) of the ninth generation showed a lymphatic metastasis consisting of simple alveoli and containing cysts. From the wall of one such cyst an outgrowth of papillomatous character had taken place (Plate XI, Fig. 21). Rat 2668 (*q*) showed a combination of anastomosing branching cell-strands and small solid alveoli filled with pale cells and showing many mitoses. There were lung nodules composed of small solid alveoli of more irregular size than the air vesicles, indicating that they had been newly formed. A new formation of stroma had taken place, and irregular giant cells of the megacaryocytic type were also present (Fig. 7). A considerable rarity was the occurrence of a metastasis in the wall of a vein; and another, a growth in the lumen of a lymphatic vessel (Plate XI, Fig. 22).

Rat 2805 (*l*) of the tenth generation showed a local lesion of the ordinary alveolar type and a growth into the kidney extending from the cortex to the pelvis and invading the renal vein. The tubules had as usual been invaded and obliterated before the glomeruli. The tumor passed to the pelvis and came to lie beneath the pelvic epithelium, which was slowly replaced, but before the replacement was completed the epithelial membrane of the pelvis had undergone a considerable hypertrophy, through which it was increased several times in thickness. Ultimately it became necrotic. The growth of the tumor into the connective tissue of the pelvis caused it to surround the arteries and veins. The former escaped invasion and occlusion. One of the latter was invaded and became thrombosed. Rat 1648 (*a*) of the eleventh generation was interesting as having shown for the first time a lymphatic gland metastasis of the typical adenomatous type, although there were still combined with it solid alveoli, while Rat 2253 (*c*) showed the superficial tumor to be of the soft, pure adenomatous or acinous type. (Plate XI, Fig. 23, Plate XII, Fig. 24, Plate XVI, Photograph 6.)

The succeeding generations were made up of tumors presenting chiefly the adenomatous form, but now and again there still appeared tumors of firmer consistence, containing solid alveoli and fibrous stroma, so that up to the present time the typical adenomatous type

has not become firmly established.   Thus Rat 3762 of the twenty-first generation (d) showed a nodule the size of a pea in the sub-cutaneous tissue, composed of small acini which were located chiefly centrally, and small, solid, elongated and oval alveoli making up the larger part and contained within a fibrous stroma.   All the cells were pale and undergoing rapid mitosis.   The stroma was remarkable for its vascularity.   At the extending edge the reticulated tissue contained many small round and spindle cells.   Rat 3855 of the twenty-third generation (c) presented a subcutaneous nodule, the central part of which was of typical adenomatous, and the cortical part of fibrous and small alveolar structure.   This tumor also contained innumerable mitoses.   The adjacent striped muscle was being invaded by solid cylinders of the pale cells, which were breaking up into fragments the muscle fibers included within the alveoli or acini.   There was no remarkable increase of muscle nuclei, but the morsels of muscular tissue were surrounded by epithelioid cells and were undergoing digestion, although they were not included, properly speaking, within cells.   In a similar manner Rat 3983 of the twenty-sixth generation (a) showed a local tumor made up of acini and solid alveoli, which was invading the voluntary muscle.   The cells composing the tumor at the point of junction with the muscle exhibited a diffuse alveolated growth in which the distinction between epithelioid cells and stroma was indistinctly marked.   On the other hand, the muscle was undergoing the same form of fragmentation and solution as has been just described.   Rat 3901 of the twenty-fourth generation (c) contained a subcutaneous nodule, which, while considerably degenerated, was an excellent example of a branching or papillary adenomatous tumor.   The stroma consisted of a delicate fibrous tissue arranged in folds and projections, showing secondary branching, which were everywhere covered with a layer of cells, one or more in thickness, of the epithelioid type, and sometimes of columnar form.   At the periphery of the nodule next to the capsule, the branching was less obvious, while acini of the typical form were present.   Specimens of this tumor stained by Mallory's phosphotungstic acid method showed extremely well the differentiation between stroma and large cells, and brought out strikingly the branching character of the large papilliferous alveoli.   Rat 3917 of

the twenty-fourth generation (*d*) presented another example of the branching papillomatous tumor. In this instance the stroma formed merely a small part of the mass of the tumor, so that by far the greater part was composed of epithelioid cells. It was towards the center of the mass, where the degeneration was advanced, that the papillomatous character was especially pronounced, while in the periphery the branching was more confined, and large solid alveoli, as well as smaller acini, were produced. Towards the center the islands of epithelium were preserved, in the midst of a soft degeneration, but these preserved cells in all cases surrounded the small vessels, the lumina of which were open. Everywhere in the tumor, and even in the islands mentioned, mitosis was going on most actively. Rat 3925, twenty-fourth generation (*c*), was an example of a subcutaneous tumor much degenerated, showing both simple acini and simple solid alveoli, and transitions of one to the other. Rat 3970, twenty-fifth generation, showed a subcutaneous nodule composed partly of acini and partly of solid alveoli, both containing pale cells. The lung of this rat contained a large nodule. This was necrotic with the exception of the extending edge, which passed without sharp demarcation into the adjacent congested lung tissue. The extension was made by means of blunt, solid outgrowths of the main tumor mass into the adjacent air vesicles, and there was an abundant increase in the interstitial tissue of the lung. The diffuse character of the proliferation made it somewhat difficult to determine the tumor nature of the nodule. Rat 4080 of the twenty-eighth generation (*c*) showed a circumscribed pulmonary metastasis composed of pale cells occupying the pulmonary alveoli and completely filling them. There were local areas of degeneration just beginning, with emigration of leucocytes and fragmentation of nuclei, but there was absence of typical acinous arrangement.

INTRA-TESTICULAR INOCULATION AND RETROGRESSION.

In the course of the inoculations several attempts were made to produce intra-testicular growths, with the idea of causing a general lymphatic dissemination of the peritoneum through that means. In all the experiments except one the result was a circumscribed necrosis

of the testicle, with more or less new formation of fibrous tissue, but no tumor growth. In one experiment tumor nodules were produced. This was in the eighth generation. The plan was to make emulsions of the tumor in salt solution and to inject them into the testicle. In the successful experiment two rats developed tumor nodules the size of small shot, just beneath the tunica of the testis and penetrating between the tubules. It was only in the periphery of the nodule that preserved, multiplying tumor cells, lying in a fibrous stroma, were observed. The centers even of these small nodules were necrotic, and consisted partly of degenerated tubular elements. There was no dissemination of the tumors.

Attention has repeatedly been drawn to examples of tumors which showed an unusual amount of fibrous tissue. Fibrous tissue is produced in two ways: (1) It is constantly produced at the edge of the growing tumors, in the course of the formation of the stroma and the capsule. It, however, is also produced in the interior, as a result of degeneration of the epithelioid elements. In growing tumors the central fibrosis comes to be quickly associated with coagulative necrosis of the tumor, which affects first the remains of the epithelioid cells and next the fibrous tissue. Much of this fibrous tissue is hyalin in structure, so that its true character may be more or less masked. (2) The second set of conditions producing fibrous transformation of the tumor is the one which we wish especially to refer to in this place. It occurs in the course of the retrogression of the tumors, and consequently is found in small as well as in larger nodules. In these cases the fibrosis tends to be unassociated with the coagulative necrosis. The fibrillation of the tissue also appears to be preserved and the epithelioid elements to be contained in contracted, narrow, often linear spaces within the tissue. The number of these cells is greatly reduced, being smallest in the central parts where the fibrosis is oldest. By compression they are eventually entirely destroyed through atrophy, in the course of which the nuclei become contracted and pyknotic, and the protoplasm, which takes on a deeper eosin-staining than it does normally, is reduced to a mere rim. · It is the peripheral portion which contains the least fibrous tissue and the most epithelioid cells, but there is a striking absence of mitosis. Briefly expressed, retrogression is associated

with an increased fibrosis, a reduced mitosis, and a gradual and ulti-mately complete shrinkage of the tumor. The removal is not, apparently, through the action of leucocytes. At least, there does not go on any gross destruction by means of these cells. In the end the fibrous tissue is as completely removed as the epithelioid ele-ments, and nothing remains in the local tissues to show the presence, at a previous time, of the tumor.

THE ELEMENTS FROM WHICH THE TUMORS ARE DERIVED.

In order to determine roughly the elements upon which successful implantation of the tumor depended, several methods were em-ployed, the results of which can be summarized briefly.

Emulsions of the tumor prepared in salt solution were filtered through unglazed porcelain, and the filtrate injected into the abdom-inal cavity and beneath the skin of the rat. In no case did a tumor develop.

Emulsions of the tumor in salt solution were filtered through sterile filter paper and injected into rats in the same manner, but in no case did a tumor develop.

Emulsions of the tumor in salt solution were passed merely through sterile gauze, and the fluid, carrying minute fragments of the tumor, was injected in the same manner into other rats, but never gave rise to tumors. On the other hand, it has been proven that similar small fragments of the tumor, when not injured by the medium in which they are suspended, can give rise to the production of tumors. This has been shown by the successful results of the inoculations of ascitic fluid containing microscopic masses of tumor cells (see page 21).

With a view of excluding, or, indeed, of confirming the presence of microscopic organisms in the tumor, sections of young growing tumors were stained with all the usual aniline dyes in the various ways employed to show bacteria or protozoa, were impregnated with silver nitrate according to Levaditi's method of staining spirochætæ, and were examined in the fresh condition with the ordinary high powers of the microscope and with the special apparatus for dark field illumination, but nothing was ever seen that was suggestive of any other structure than the proper cellular elements of the tumor.

Aerobic and anaerobic cultures were made from young undegenerated and older degenerated tumors, but they never yielded any growth of bacteria.

THE ACTION OF PHOTO-DYNAMIC SUBSTANCES ON THE TUMOR.

Experiments showed that when bits of the tumor of some size were kept immersed for a brief period in physiological salt solution, tumors could still be reared from them, but they also showed that the minute fragments which would pass through the meshes of ordinary gauze were no longer able to yield tumors when exposed to the action of this solution.   The failure in the latter experiments was attributed to the poisonous effects of the sodium chloride; and it was proved that Ringer's solution, in which these poisonous effects are balanced by the use of the calcium, was less injurious to the tumor than salt solution alone.   A better, because less injurious, fluid for suspending the tumor proved to be rat serum heated to 54° C., but in making the transplantations the fragments of tumor which were introduced were not permitted to come into contact with any extraneous fluid whatever.   In this way the best results were obtained.

An effort was made to influence the growth, persistence and transplantability of the tumor through the action of photodynamic agents.   We believed at one time that we had secured such an action by means of eosin and Bengal red, but later study of the action of these elements led us to the conclusion that the early results which we had obtained and supposed to be due to the action of the photodynamic anilines was attributable to other causes.   We shall give a table illustrating the irregularity of the results of transplantation of a single specimen of the tumor, illustrating particularly the great variation in the number of retrogressions taking place in a series. The method was to expose minute fragments of the tumor to the action of the various solutions given in the table.   It was intended that the exposure should be one hour, but the large number of animals to be inoculated required that the exposure extend from one to three hours, since a part of the inoculations were not completed until the expiration of that time.   However, the light exposures were all limited to one hour.

	No.	Tumors, Per Cent.	Retrogressions, Per Cent.
Controls	10	10 or 100	3 or 30
NaCl : light	10	10 or 100	3 or 30
NaCl : dark	10	10 or 100	4 or 40
NaCl eosin 1–1000 : light	10	9 or 90	3 or 33
NaCl eosin 1–1000 : dark	10	9 or 90	8 or 88
NaCl Bengal 1–1000 : light	10	10 or 100	10 or 100
NaCl Bengal 1–1000 : dark	9	8 or 88	1 or 12.5
Ringer's : light	10	7 or 70	6 or 85.7
Ringer's : dark	10	9 or 90	2 or 22
Ringer's eosin 1–1000 : light	10	10 or 100	3 or 30
Ringer's eosin 1–1000 : dark	9	9 or 100	3 or 33
Ringer's Bengal 1–1000 : light	9	9 or 100	2 or 22
Ringer's Bengal 1–1000 : dark	10	9 or 90	2 or 22
Serum : light	10	10 or 100	2 or 20
Serum : dark	7	7 or 100	2 or 28
Serum eosin 1–1000 : light	10	10 or 100	5 or 50
Serum eosin 1–1000 : dark	10	10 or 100	4 or 40
Serum Bengal 1–1000 : light	10	9 or 90	1 or 11
Serum Bengal 1–1000 : dark	10	8 or 80	5 or 62

An explanation of the table brings out the fact that the tumor under the influence of the anilines tends to give a larger number of retrogressions than in the absence of the anilines, and also that exposure to sunlight for one hour is possibly to some small extent injurious, but no general deductions can be made from the experiment, and we publish this final table in order to set ourselves right on this point.

MODE OF DEVELOPMENT OF THE TRANSPLANTED TUMOR.

Leo Loeb, and later Bashford, and since then others, have made careful observations on the manner of development of the transplanted fragments of tumors.   We have ourselves studied the manner in which the fragments of this tumor undergo development in a new animal.   Indeed, we have studied the changes in the transplanted fragments which occur not only in the rat but also in the mouse and the guinea-pig.   We owe to Ehrlich the important observation that a tumor fragment introduced into a species from which it was not developed, as from a mouse into a rat, would undergo a limited development in the new species, but would never attain a considerable size or become organically attached to the individual of the new species, or be transplantable from one to another of this new species.   On the other hand, such living fragments can be

transferred successfully at some periods from the new to the original species, and then be transplanted further.

We have found in common with Loeb and Bashford that the greater part of the tissue transplanted undergoes necrosis, and that the new tumor is produced from a small number of surviving cells. Our observations were made with the usual fragments transplanted by us and also with a somewhat finer emulsion (made by comminuting the living cortical parts of the tumor by means of a Haaland grinder) deposited beneath the skin in the region of the axilla. A series of rats, mice and guinea-pigs were inoculated on the same day with fragments and emulsion from one source. They were chloroformed at intervals of twenty-four hours; the implanted tumor with the surrounding tissue was removed, preserved and hardened in Zenker's fluid, and sections stained in hematoxylin, eosin and iron-hematoxylin were prepared.

FRAGMENTS IN THE RAT.

The fragments of tissue in the emulsions were several times smaller than the morsels which were introduced by means of the inoculating needle; fewer of them survived, and their development was on the whole much less rapid. The essential process of growth was, however, identical in the two series.

At the end of twenty-four hours considerable portions of the transplanted tissue fragments had become necrotic. The tissue of the fragments, excepting the surviving cells, to be described, presented appearances similar to those which tissue permitted to undergo autolysis at the body temperature outside the body would be expected to show. The interstitial tissue showed few deformed and fragmented nuclei; the fibers were indistinct and more or less fused together; and the epithelioid cells occupying small spaces in the latter tissue were shriveled, with irregular outline, the protoplasm staining deeply in eosin, the nuclei of irregular form and unequal staining powers, and in process of disintegration. The fragments were everywhere, as was to be expected, surrounded by a fibrinous and leucocytic exudate, and a certain number of leucocytes had penetrated within them. Within certain of the fragments a number of

epithelioid cells had survived. These cells might be at the periphery or they might be in the interior of the fragments, sometimes single and sometimes several in a given space. They differed greatly from the degenerating and disintegrating cells, particularly in respect to the behavior of their nuclei, which retained the normal form and distribution of chromatin, although in point of intensity of staining they tended to be hypochromatic. For the most part the cells appeared merely to be survivals, but now and again nuclear changes suggestive of mitosis were met with. However, in view of the fact that the chromatic filaments were somewhat irregular in thickness and arrangement, it cannot be said that those changes actually led to cell multiplication at this early period. It should be mentioned that in some fragments the old blood vessels still remained morphologically unchanged at the end of this period.

At the expiration of forty-eight hours the effects of the transplantation were still more obvious. The stroma and the greater part of the epithelioid cells and the blood vessels in the transplanted morsels had all progressed still further in respect to the disintegrative changes. On the other hand, the surviving epithelioid cells had, either by contrast or actuality, become more sharply demarcated from the surrounding elements. Where they were contained within the interior of the fragments, their number had not greatly increased, although apparently there had already been some increase in number; the nuclei had become richer in chromatin, the surrounding protoplasm clearly outlined, and mitosis had not only become more frequent than in the twenty-four hour period, but the chromatin filaments presented a regular arrangement and uniform thickness suggestive of normal mitosis. At the periphery of some of the fragments the epithelioid cells had clearly multiplied, so that they had come to form a superficial layer, one, two or three cells deep in some places. These cells lay in contact with the inflammatory exudate, and some of them apparently had wandered into the exudate. Mitoses also occurred in them, and even in separated cells surrounded by the exudate. Thus far no organic connections had taken place between the fragments and the surrounding tissue, although in the latter an active proliferation and new formation of capillaries had begun. On the other hand, elongated cells of fibroblastic or angio-

blastic nature had begun to enter the fragments at the periphery, adjoining the host tissues.

After three days the conditions had altered greatly. The fragments had not increased perceptibly in size, but those lying adjacent to the host tissues had become attached through capillary connections with the tissues of the living animal. The capillaries had pushed in various directions and some of considerable size had already formed channels in the interior. The medium in which they lay was apparently nothing else than the degenerated original stroma, and there had been no unmistakable restoration of living interstitial cells. All the cells which had wandered in were in connection apparently with the capillaries. On the other hand, the epithelioid cells had increased greatly, mitosis having gone on rapidly. The cells now formed small groups chiefly, although sometimes single ones were separated from the others. The active changes in the vascularized fragments were greatly in advance of those not already vascularized. Moreover, those fragments or portions of fragments in which the epithelioid cells had not begun to multiply, nor the capillaries to penetrate, had by this time been invaded with large numbers of leucocytes, which were softening and dissolving the necrotic tissue. Finally, in certain fragments there had apparently been beginning multiplication of the epithelioid cells, which had not become vascularized, and which were undergoing a secondary degeneration and death.

At the end of the fourth day the conditions in the vascularized peripheral fragments represented merely progression of the third. In other words, the vascularity having increased, these fragments were now entirely occupied by capillary vessels and proliferated epithelioid cells, while the fragments which were more interior were less advanced in this condition, and might show mitosis of epithelioid cells still going on, although no capillaries were yet visible in the stroma, which was quite completely necrotic. At the same time, occasional islands of epithelioid cells remained alive and were proliferating slowly.

The subsequent progress was rapid. The groups of epithelioid cells continued to increase in number and to expand in size, and coincidentally a fibrillated tissue appeared between them and the

capillaries. At the end of seven days the fragments had become completely vascularized, all of the evidences of acute inflammation had disappeared, the volume of the fragments had increased several fold, and they had formed an indissoluble union with the tissues of the host. At the junction of the two, a small-cell granulation tissue formed a sort of capsule. The positions of the original fragment could still be made out through the remains of a central, paler, partially degenerated, and not completely substituted mass of epithelioid cells and young capillaries, with here and there polynuclear leucocytes and beginning fibrillated tissue. What was particularly striking was the close union of the epithelioid cells and the young connective tissue. This union was so close that alveolation in the proper sense of the term could not be spoken of. In addition, there occurred not infrequently multinucleated protoplasmic masses giving rise to giant cells and large single cells with giant nuclei.

By the ninth day the differentiation between the fibrous stroma and the epithelioid elements had progressed so far that as the connective tissue became more fibrous it left the epithelial cells more sharply demarcated. On the eleventh day this demarcation was still more pronounced, although the condition was not as yet one of definite alveolation. At this time the remains of the original fragment occupying the central portion of the enlarged nodule was still evident. It consisted of a hyalin mass enclosing small groups or single epithelioid cells not in the best state of preservation. On the fourteenth day the nodule was still further increased in size, the connective tissue had become more pronouncedly fibrous, but the epithelioid cells were not so strictly confined as to produce a typical alveolated structure. On the other hand, on the fifteenth day the fragment was quite characteristically alveolated.[5] The connective tissue now formed a definite fibrous stroma enclosing alveoli, chiefly solid but sometimes presenting spaces and, therefore, acinous, of epithelioid cells, which were multiplying rapidly by mitosis. These alveoli were shown, especially in the preparations stained with iron-hematoxalin, to be of irregular size and form and everywhere intersected by the fibrous stroma. In that respect at this period the

[5] This change is one of degree only and would doubtless vary in different experiments.

alveolation was not by any means so regular as was found in some later nodules and as is the rule in simple carcinoma in man.

FRAGMENTS IN THE MOUSE.

The results of the introduction of fragments of the tumor under the skin of the mouse were in many respects, for the early period, similar to those in the rat. The first effect was, of course, a certain amount of inflammatory exudation, but this amount was not greater than that which took place in the rat. The changes in the fragments were also similar, which is to say that the greater part of the tissue composing them degenerated. But what remained alive at the end of twenty-four hours was a certain number of epithelioid cells on the surface or in the interior of the fragment. At the end of the first twenty-four hour period mitosis had not been noticed in the host cells, but the stroma and many of the epithelioid cells were in a state of complete necrosis, and the resistant epithelioid cells had begun to show mitosis. The formation of angioblasts and the organic connection with the mouse of the fragments also occurred on the third day, at which time there had been a great proliferation by mitosis of the epithelioid cells, particularly those lying immediately next to the tissues of the mouse, which were united to the fragments, although the mitosis was not limited to those cells. The increase in the epithelioid cells on the fifth day was considerable, and they formed groups within the homogeneous residue of the stroma, which was now in process of invasion by capillaries. On the seventh day the new vessels had penetrated deeply into the fragment; there was a striking hyperemia, and the epithelioid cells were still in process of multiplication. On the eleventh day the healing process was still more advanced, but remains of the epithelioid cells, which were, however, inactive, were still present. On the fifteenth day the vascularization was still more advanced, and had reached almost to the center of the fragments, which were occupied by masses of leucocytes undergoing fragmentation, but the specimens showed that multiplication by mitosis was still going on actively in the epithelioid cells, especially in the vicinity of the tissues of the host. The absence, therefore,

of active mitosis in the two previous periods was the result not of the exhaustion of the tissue but of accidental circumstances in connection with the transplanted fragments. Although the latest period in which active mitosis was discovered in the fragments transplanted to the mouse was fifteen days, the large number of them contained in one series of specimens of that period indicates than they might still be found under favorable circumstances even at a later period.

We have transferred fragments which have remained in the mouse for different periods back to the rat and have succeeded in rearing these fragments as late as eight days after their implantation in the mouse.

FRAGMENTS IN THE GUINEA-PIG.

Fragments transplanted to the guinea-pig aroused a somewhat greater inflammatory reaction and exudation than in the other animals mentioned. The changes which took place in the transplanted fragments did not differ essentially from those already described. While the great mass of the tissue died and showed the changes of autolysis, groups of epithelioid cells in the interior or near the surface survived. At the end of the first twenty-four hour period these surviving cells were still quiescent, while at the end of forty-eight hours they had changed significantly: they had become sharply demarcated from the rest of the tissue, were more numerous than they had been in the previous twenty-four hours, and were showing active mitotic division. Indeed, this activity was more marked than it had been under similar circumstances, either in the rat or the mouse. The multiplication was taking place in the interior of the fragments, as well as on the surface. At the latter situation the new cells had already formed a layer several deep. There was still activity, but it was less in these cells at the end of the third day, at which time there were fewer organic connections with the host than had been noted in the other animals, and many fewer new blood vessels had formed. On the seventh day the vascularization was well advanced, but not equally about all the fragments. About some the exudation was very great and had

penetrated into the interior.	Karyokinesis was, however, still going on in the epithelioid cells, although not actively, and particularly at the periphery of the fragments.	The epithelioid cells in the interior were reduced in numbers, and were degenerating.	On the ninth day the fragments had been greatly reduced in size and had become much more disintegrated, so that the demarcation of the epithelioid cells was uncertain.

SUMMARY.

The rat tumor described is to be regarded probably as an embryoma located in the seminal vesicle, from whose histological elements it was partially derived.

Originally the tumor was composed apparently of a simple tissue of imperfectly organized structure, but containing certain glandular elements derived in part from the seminal vesicle and in part from other included epithelial organs.

The included glandular elements had already undergone in the original tumor a carcinomatous development, although the carcinomatous moiety was not disseminated throughout the mass, but was confined to portions near its peripheral boundary, adjacent to the wall of the vesicle.

In the course of transplantation the simple and imperfectly organized structure of the tumor became substituted by a more complex and perfectly organized tissue, which bore resemblance to simple alveolar carcinoma.

The tumor at this time not only developed in the subcutaneous and intra-muscular tissue and the abdominal cavity, according as the grafts were made, but it gave rise to metastases through the blood current in the lungs and kidneys.

In the fifth generation the simple carcinomatous structure began to be replaced by a tissue resembling adeno-carcinoma, and coincidentally metastases appeared in the superficial and rarely in the deeper, distant lymphatic glands.

The adeno-carcinomatous form of structure became progressively more established in the succeeding generations, in process of which the stroma of the tumor changed to a delicate fibrillated tissue supporting the acini, and differing materially from the rich fibrous stroma that usually supported the simple alveoli.

The tumor had not become established as an adeno-carcinoma of the pure type by the twenty-eighth generation, at which time simple alveoli still appeared, but the stroma of the tumors bearing the latter structure was relatively delicate, and the consistence of the tumors relatively soft, in contradistinction to the earlier type, which was much firmer.

The degenerating tumors of the earlier type showed much fibrous tissue of a hyalin quality, while those of the later type showed coagulative necrosis of a soft quality.

The metastases partook more of the nature of simple carcinoma than of adeno-carcinoma, even in the later type. The tumor cells composing the metastases tended to be substituted for the epithelial cells of the lungs and kidneys and the lymphoid cells of the lymphatic glands, and merely to occupy the already prepared stroma of these organs. Hence the structure of the organs in the metastases was not greatly disturbed. When a metastasis appeared in the pleural membrane, in which fibrous tissue was readily produced, the acinous type of tumor tended to appear, and in the course of the invasion of the cardiac muscle and other solid tissues, acini also tended to be produced.

The tumor presented a high degree of invasive power, and its growth was not interrupted by muscle, fascia, bone or cartilage.

The dilated esophageal extremity of the stomach tended to become invaded. When the tumor reached the mucous membrane, ulcers developed, and implantation to the opposite surface of the mucosa sometimes occurred.

The retrogression of the growing tumors was accomplished through atrophy and degeneration of the epithelioid cells contained in the tumor and an increasing degree of fibrosis of the stroma. Ultimately all parts of the tumor disappeared completely. Nothing was discovered to indicate that the metastases ever underwent changes of the nature of those leading to retrogression of the superficial nodules.

Although the metastases were such as were produced chiefly through the blood current, it was most rare to find masses of tumor

cells in the blood vessels, unless they had penetrated them second-arily from without.[6]

The manner of development of the fragments of the transplanted tumor showed the large epithelioid cells to be the proper elements of the tumor.   All other tissue elements succumbed, and the reproduction of the tumor depended upon the proliferation by mitosis of these cells, the stroma for which was supplied by the tissues of the new host.

There was no essential difference in the manner of survival of grafts introduced into rats, mice and guinea-pigs, except that in the last two species the process of multiplication of the epithelioid cells came to an end in a few days, in spite of the vascularization of the transplanted fragments, which at first promoted proliferation.

The number and location of fragments surviving in the rats depended in part on the rapidity and degree with which the vascularization took place.   The first stages of proliferation did not depend upon the vascularization, but unless new vessels had entered the fragments by the third day, the epithelioid cells already produced tended to degenerate rather than to proliferate further.

A highly glandular organ such as the testicle proved not to be very suitable for the tumor implantations.

Salt solution proved to be injurious to the tumor tissue, especially when it was in a state of fine division.   The tumor was acted on injuriously by sunlight, and this injurious action appeared to be somewhat intensified by the presence of certain photo-dynamic anilines.

Filtered tumor emulsions and even strained tumor emulsions in salt solution did not produce new tumors upon inoculation, while small aggregates of the tumor cells, such as occur in ascitic fluid, did set up the formation of new tumors.

No microörganisms were recognized in the tumor, and no bacterial cultures were obtained from non-ulcerated tumors.

[6] We are indebted to Miss Menten for a very careful study of large numbers of serial sections of the lungs of rats, in which metastases were present, and in which no visible metastases could be seen. She succeeded in a single instance in finding a small mass of tumor cells within a blood vessel, unassociated with a developed metastasis, and this was in a lung already the seat of metastatic nodules.

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.
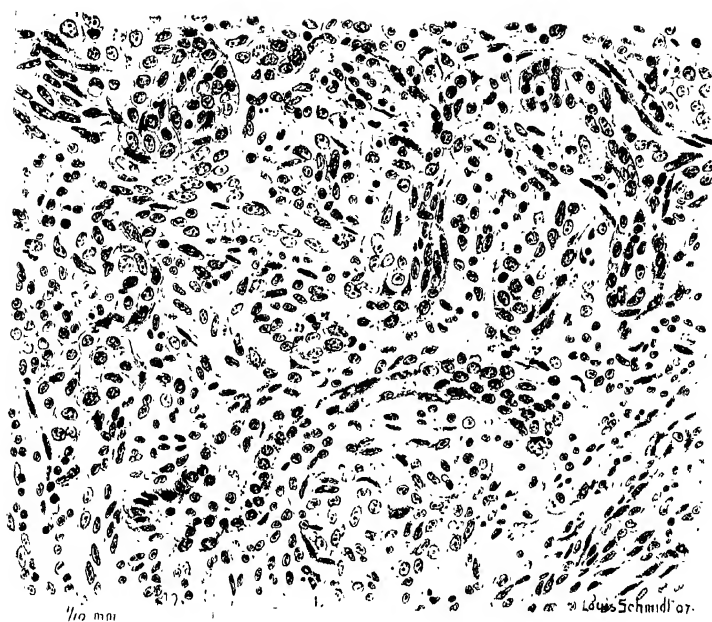
FIG. 6.
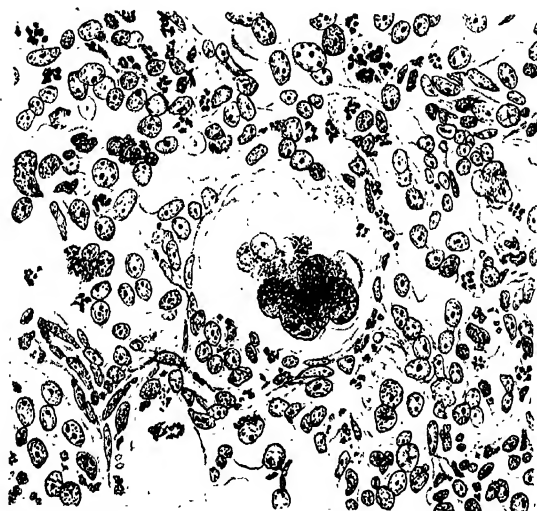
FIG. 7.
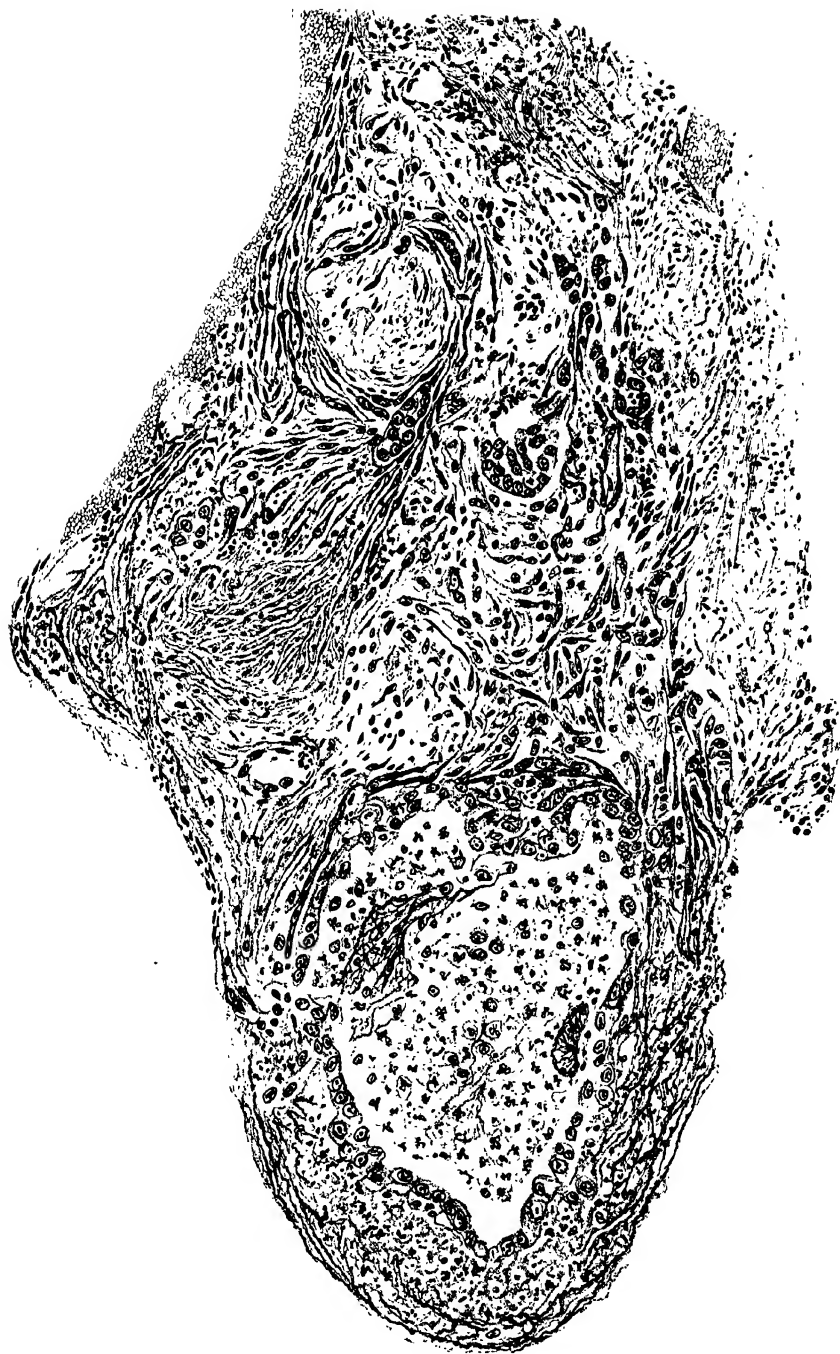
Fig. 8.

FIG. 9.

FIG. 10.

FIG. 11.

FIG. 12.

1/10 mm
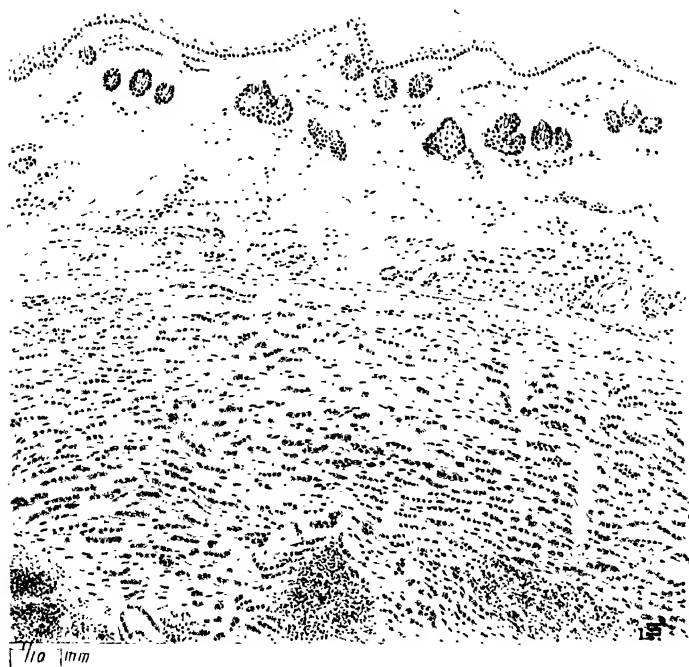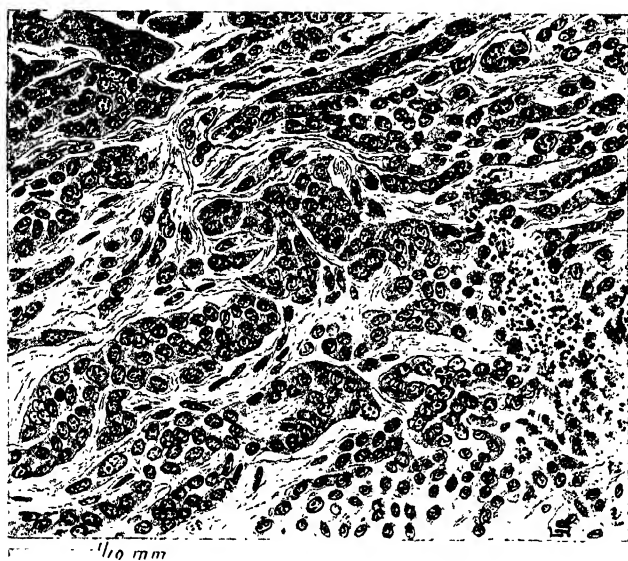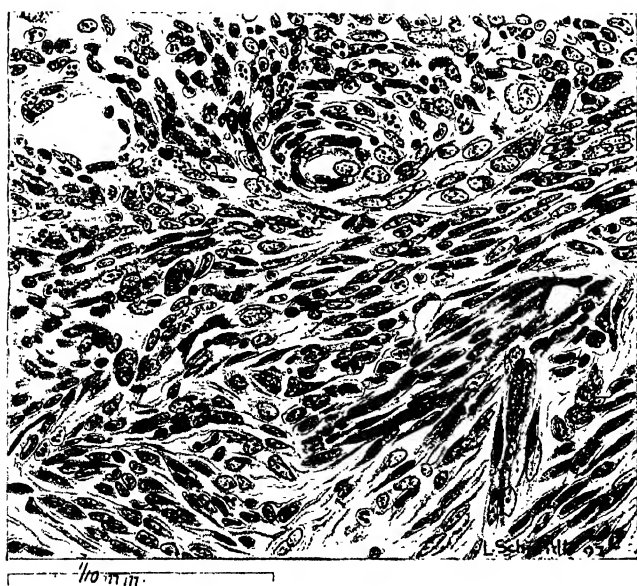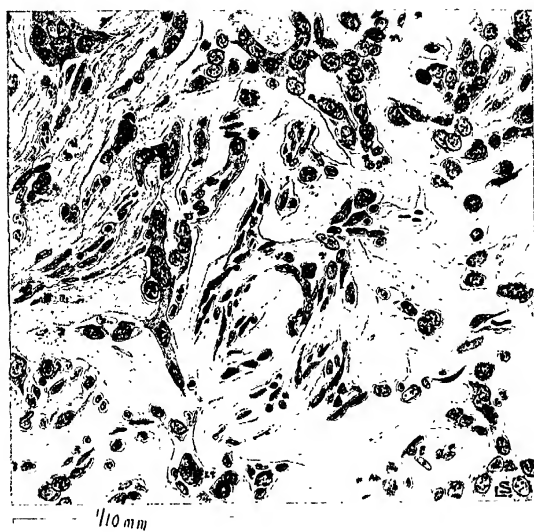
Fig. 13.

Fig. 14.
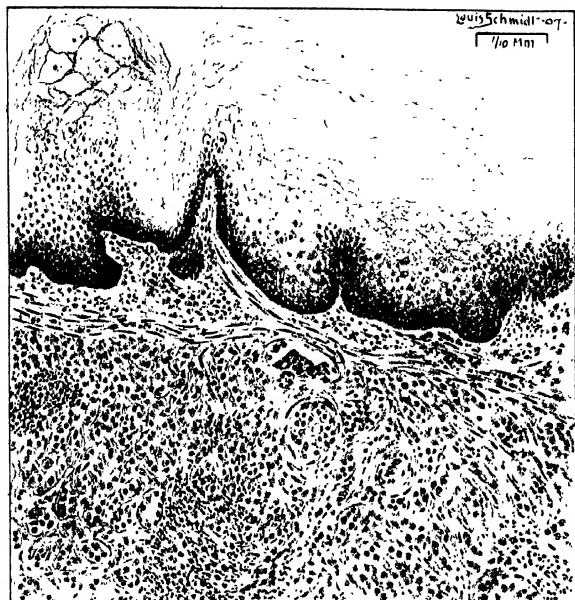
FIG. 15.

FIG. 16.
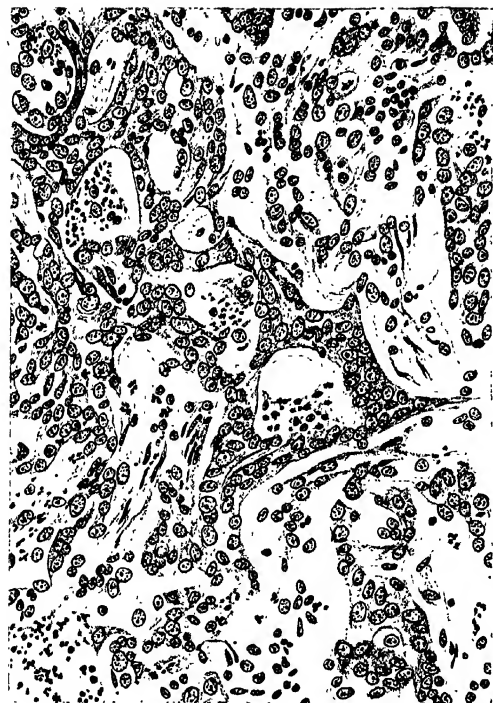
FIG. 17.
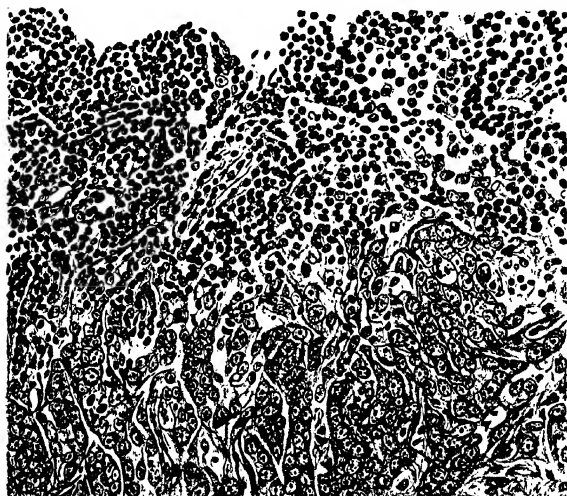
FIG. 18.
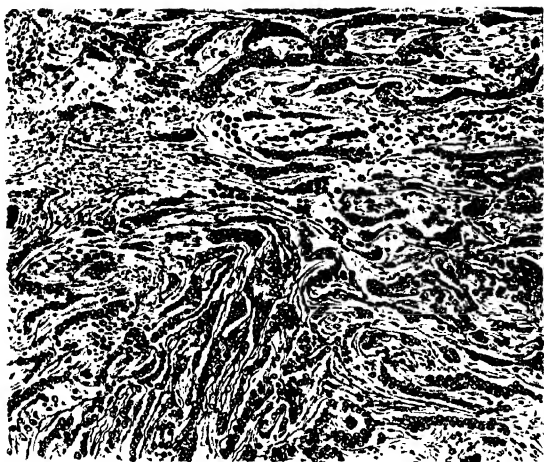
Fig. 19.

Fig. 20.
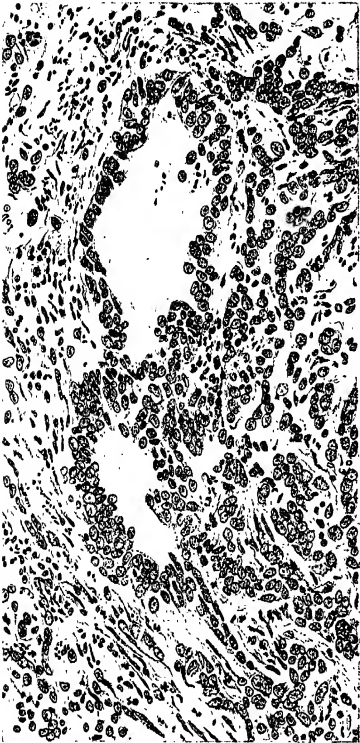
FIG. 21.

FIG. 22.

FIG. 23.
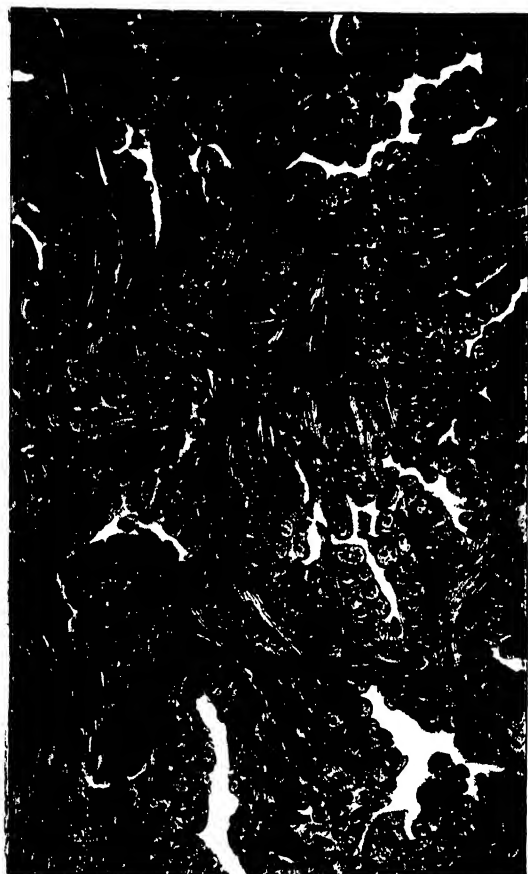
FIG. 24.
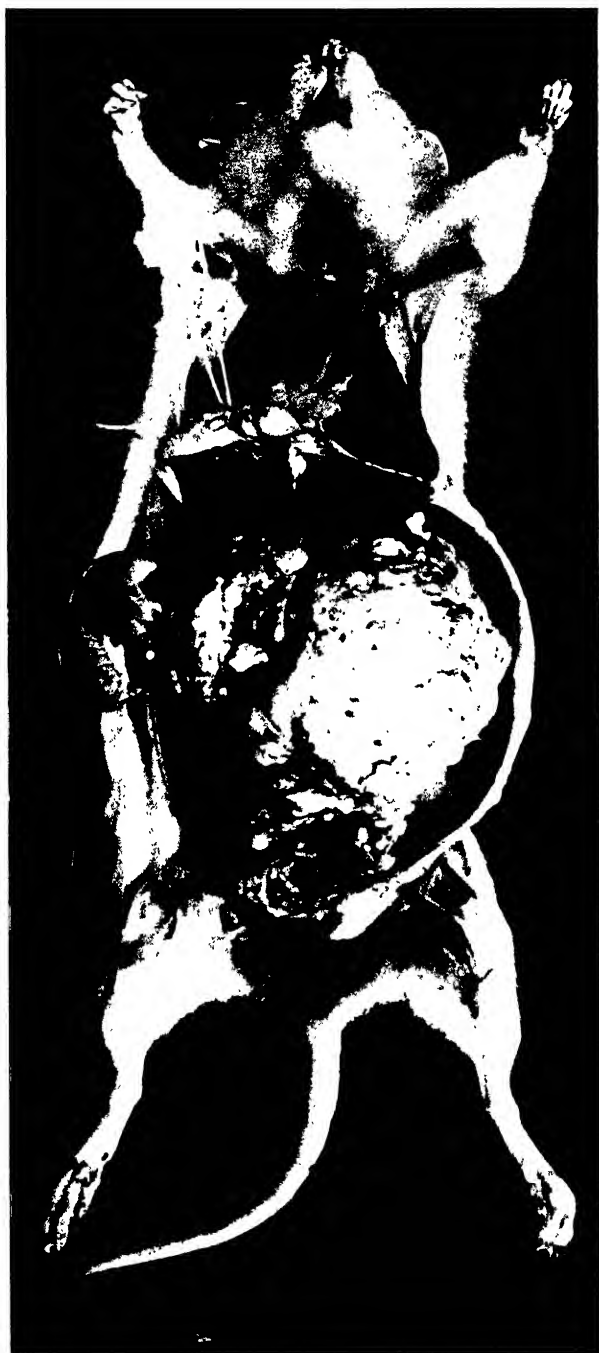
PHOTOGRAPH I.

PHOTOGRAPH 2

PHOTOGRAPH 3.

PHOTOGRAPH 4.

PHOTOGRAPH 5.

Photograph 6.

EXPLANATION OF PLATES.

PLATE I.

FIG. 1. No. 3 eye-piece; 6 objective. Showing the relation between the spindle and polymorphous cells occurring over considerable areas of the tumor.

FIG. 2. No. 3 eye-piece; 6 objective. Glandular tubule included in the tumor undergoing a typical proliferation or being dissected by ingrowth of the tumor.

FIG. 3. No. 3 eye-piece; 3 objective. Dilated glandular tubule enclosed in hyalin connective tissue adjacent to alveoli enclosing epithelial cells, probably derived from the tubule.

PLATE II.

FIG. 4. No. 3 eye-piece; 3 objective. Junction of the seminal vesicle, showing dilated glandular spaces, with the tumor, and typical carcinomatous alveoli possessing lumena.

FIG. 5. No. 3 eye-piece. 3 objective. Branching tubular formation or duct adjacent to a glandular mass possessing structures of a serous or mucous gland.

PLATE III.

FIG. 6. No. 3 eye-piece; 6 objective. Irregular carcinomatous alveoli embedded in the stroma, containing spindle and other cells resembling the epithelium-like polymorphous cells of the alveoli.

FIG. 7. No. 3 eye-piece; 6 objective. Pulmonary tumor nodule of alveolar structure, showing a mononucleated giant cell of the megacaryocytic type.

PLATE IV.

FIG 8. No. 3 eye-piece; 6 objective. Invasion of the heart wall and coronory sinus by the tumor.

PLATE V.

FIG. 9. No. 3 eye-piece; 6 objective. Stomach opened to show the ulcerated tumor masses in the esophogeal segment. The elevated, undulating white line shows the demarcation between the esophogeal and gastric mucosa.

FIG. 10. No. 3 eye-piece; 3 objective. Subcutaneous tumor nodule showing the relation of the young alveoli to the stroma. A definite demarcation separates the tumor from the cutis.

PLATE VI.

FIG. 11. No. 3 eye-piece; 6 objective. Showing the usual structure of the tumor in the stage of carcinoma simplex.

FIG. 12. No. 3 eye-piece; 6 objective. From the edge of a recent rapidly growing graft showing the original development of spindle cells and small included masses of polymorphous cells which have not yet arranged themselves into definite alveoli.

PLATE VII.

FIG. 13. No. 3 eye-piece; 6 objective. Hyalin transformation of the tumor in the course of retrogression showing the persistence of small groups and masses of the polymorphous cells within the hyalin connective tissue.

FIG. 14. No. 3 eye-piece; 3 objective. Invasion of the splenic vein by the tumor which presents a typical tubular alveolar structure. The vein is occluded by a thrombus which is itself becoming invaded with tumor acini.

PLATE VIII.

FIG. 15. No. 3 eye-piece; 3 objective. The invasion of the submucous and subepithelial layer of the esophageal segment of the stomach by the tumor which shows distinct alveolation. Beginning degeneration of the superficial epithelial cells.

FIG. 16. No. 3 eye-piece; 3 objective. Invasion of the vertebral column. The alveolar tumor in the section is adjacent to the cartilages and bony structures.

PLATE IX.

FIG. 17. No. 3 eye-piece; 6 objective. Pseudo-acinar excavation of the alveoli.

FIG. 18. No. 3 eye-piece; 6 objective. Invasion of the regional lymphatic glands by the alveolated tumor. The stroma which is slight is furnished by the gland.

PLATE X.

FIG. 19. No. 3 eye-piece; 3 objective. Atypical carcinoma simplex in which the alveoli are linear and bent in several directions.

FIG. 20. No. 3 eye-piece; 3 objective. Invasion of the large peripheral nerve by the tumor.

PLATE XI.

FIG. 21. No. 3 eye-piece; 6 objective. Lymphatic metastasis undergoing cystic degeneration and showing papillary outgrowths in the wall.

FIG. 22. No. 3 eye-piece; 6 objective. Invasion of a lymphatic vessel by the polymorphonuclear cells of the tumor.

FIG. 23. No. 3 eye-piece; 6 objective. Typical soft adeno-carcinomatous form of the tumor.

PLATE XII.

FIG. 24. No. 3 eye-piece; 6 objective. The same stained with Mallory's aniline-blue to show the relation between the stroma and epithelial cells.

PLATE XIII.

PHOTOGRAPH 1. (Rat. 47, p. 23.) Large, omental nodule, multiple, small omental and mesenteric nodules, and nodules in the diaphragm, all developing from a single fragment introduced into the peritoneal cavity.

PLATE XIV.

PHOTOGRAPH 2. (Rat 46, p. 24.) The tumor growing·about and into the cardia of the stomach where a cup-shaped ulcer is produced.

PHOTOGRAPH 3. (Rat 231, p. 26.) A large tumor metastasis in the axillary gland and smaller metastasis in the retroperitoneal and other lymphatic glands, and a growth into the pleural cavity.

PLATE XV.

PHOTOGRAPH 4. (Rat 231, p. 26.) Metastasis in the axillary and retroperitoneal glands, and in the lungs.

PHOTOGRAPH 5. (Rat 424, p. 27.) The development of multiple nodules from an intraperitoneal injection of ascitic fluid containing microscopic tumor fragments.

PLATE XVI.

PHOTOGRAPH 6. (Rat 2253, p. 42.) The histological appearance of the typical, soft, pure, adenomatous and acinous growth.

THE BIOLOGY OF A MIXED TUMOR OF THE RAT

By J. W. JOBLING, M.D.

THE BIOLOGY OF A MIXED TUMOR OF THE RAT.

BY J. W. JOBLING, M.D.

(*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*)

The tumor, of which the pathological anatomy has just been fully described, has been studied under a number of artificial conditions with a view of establishing certain facts regarding its biology. The more extensive biological studies of tumors in the lower animals made up to the present time have been carried out on mice, and to a much less extent on dogs and rats. Indeed, there does not exist in the literature such an exhaustive study of a tumor in other animals comparable to many studies which have been carried out in connection with the transplantable tumors of mice. It is, therefore, of some interest to ascertain to what extent the general facts established for tumors of mice are especially true for the transplantable tumors of other species of animals, so that in the description to follow, along with certain observations applying peculiarly to this tumor, others will be described which are based on previous experiments of a similar nature made upon mice.

GROWTH AND RETROGRESSION.

The phenomena of growth of the tumor were studied under a number of artificial conditions. The first of these which we are to consider refers to the transplantation generations, and particularly to the percentages of successful implantations and the percentages of retrogressing tumors in the several series. We will discuss these points in relation to the first twenty-five transplantation generations.

Beginning with the third generation, the number of rats inoculated for each one ranged from 13 to 125. The lowest per cent. of successful inoculations was observed in the third generation, since only four animals of the thirteen inoculated developed tumors, and of these four, two finally disappeared by retrogression. The age of

the tumor used for the inoculations was 132 days, and as no careful selection was made of the fragments transplanted, this factor doubtless played a part in the result. The tumor employed for inoculation in the fourth generation was forty-eight days old and measured one centimeter in diameter. Of the 35 rats inoculated, 24, or 68.5 per cent., developed tumors, of which 4, or 16.6 per cent., later underwent retrogression. From this time on the tumors chosen for use in inoculating the other rats were about one centimeter in diameter, although they were of very different ages. The youngest tumor employed was 23 days old, and the oldest, 71. In the seventh generation the number of successful inoculations reached 96.6 per cent. That is, of 30 rats inoculated, 29 developed tumors, of which 6, or 20.7 per cent., subsequently disappeared. From this time on, in spite of certain fluctuations, the percentage of successful inoculations remained high, being usually 90 per cent. or over, and falling once only, namely, in the twenty-third generation, to 68.4 per cent. In the twentieth generation all of the 29 rats inoculated developed tumors.

Along with the development of increased power of growth in the inoculated rats, there went a diminished tendency to spontaneous absorption of the tumors. Until the twelfth generation the number of retrogressions was high and reached sometimes 52.7 per cent. in the tenth generation, and 32 per cent. in the twelfth. After this, the percentage of retrogressions tended to remain below 10 per cent.; once it fell to 2 per cent., and once, namely, in the twenty-second generation, it rose as high as 23.6 per cent.

That the tumor is a slowly growing one is readily seen from the length of time the inoculated animals survived the implantations and the relatively small size which the tumors attained at the time of the death of the rats. The tumor causes death probably in one of three ways: first, through ulceration of the skin and secondary infection; second, through invasion of vital parts; and third, through metastasis. In respect to the degree of local development, the tumor stands far behind the Jensen and Lewin rat tumors, which we have also had the opportunity of studying. After the implantation of fragments the earliest evidences of increase in size were obtained on the seventh to the tenth day. From that period the growth is pro-

gressive and quite uniform, but not rapid, and the tumors reach sometimes a considerable size, measuring not infrequently three centimeters in their greatest diameter.

ACTIVE IMMUNITY.

It is now established, chiefly as a result of the accurate studies of Gaylord and Clowes,[1] Jensen, Ehrlich,[2] Bashford,[3] and still others, that mice which first developed transplantation tumors, subsequently undergoing absorption possess a high degree of refractoriness, comparable perhaps to a state of active immunity to subsequent inoculations of similar or even diverse tumor fragments. We observed in conformity with this established fact that the rats, which, having developed tumors, subsequently lost them through absorption, showed a considerable degree of refractoriness upon reinoculation with the same kind of tumor fragments. An experiment was then made to determine whether this state of resistance or immunity, so-called, was a fixed or enduring quality or whether it was subject to quantitative changes produced through lapse of time. The following tabulation bears on this question:

TABLE I.

Influence of Time Period on Reinoculability of "Immune" Rats.

Elapsed period since disappearance.	Number reinoculated.	Number developing tumors.	Number in which tumors disappeared.
First 30 days	15	1	1
30 to 60 days	16	5	0
60 to 90 days	26	4	0
90 to 150 days	4	0	
Totals	61	10	1
Control	80	69	21

Table I shows, first, that the state of refractoriness left by the retrogression of growing tumors is not perfect, but is of high degree; and it further indicates that this degree is greatest immediately after the disappearance of the tumors, and becomes reduced subsequently

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[1] Gaylord and Clowes, *Med. News,* 1905, lxxxvii, 698.

[2] Ehrlich, *Arb. a. d. k. Inst. f. exper. Therapie,* 1906, No. 1, 77.

[3] Bashford, *Proc. of the Royal Soc.,* Series B, 1907, lxxix, 164.

by mere lapse of time.   Within a period of 90 days after the retrogression of the tumor in 61 rats, 10, or 16.5 per cent., proved reinoculable with a tumor which yielded in the control animals 86.2 per cent. of tumors.   Moreover, of the 10 tumors developing in the refractory rats, only one was afterwards absorbed, and that was in a rat coming within the first thirty-day period since the original absorption.   Among the control rats, on the other hand, the retrogressions were considerably higher, and equalled 31.8 per cent.

It is significant that on a second attempt to implant successfully tumor grafts in 13 rats that had resisted a first reinoculation, from 30 to 150 days previously, none were successful.   The control rats of this series gave 75 per cent. of successful implantations.

NATURAL REFRACTORINESS.

It has been repeatedly observed by all who have studied transplantable tumors that animals which resist implantation of tumor grafts are not wholly immune, but are merely refractory, and can often be successfully inoculated with a similar tumor of higher virulence, or, as is sometimes stated, with greater capacity for growth. We were interested not only in confirming this result with the rat tumor, but also in ascertaining to what extent the effect of the first unsuccessful implantation tended to add to the natural refractory state of the animals.   Having ascertained that mere lapse of time was attended by diminution of this refractory state in some rats (Table I), a test similar to that employed in them was now applied to the rats which resisted a first inoculation.   The result is shown in Table II.

TABLE II.

Influence of Time Period on Inoculability of Negative Rats.

Elapsed period since unsuccessful inoculation.	Number reinoculated.	Number and per cent. developing tumors.	Number in which tumors disappeared.
First 30 days	17	2 (11.7)	0
30 to 60 days	60	31 (51.6)	12
60 to 90 days	62	33 (53.2)	3
90 to 120 days	47	27 (57.4)	3
120 to 150 days	14	5 (35.7)	1
Totals	200	98 (49)	19
Controls	80	69 (86.2)	21

But before discussing the results, another tabulation (Table III) will be given, since it expresses the effect of a third implantation of tumor fragments in rats which had resisted two previous inoculations.

TABLE III.

Influence of Time Period on Inoculability of Negative Rats.

Elapsed period since second negative inoculation.	Number reinoculated.	Number and per cent. developing tumors.	Number in which tumors disappeared.
First 30 days	18	2 (11.1)	1
30 to 60 days	17	3 (17.6)	0
Total	35	5 (14.2)	1
Control	20	15 (75)	6

These tabulations indicate clearly that failure in respect to the tumor implantations depends upon factors which are not controlled entirely by the animal subjected to the inoculation, or even by the fragment of tumor implanted, but represents the joint result of the action of both sets of conditions on each other, namely, those inherent in the animal and those inherent in the tumor. The virulence or the capacity for growth of the tumor used to inoculate the negative animals at the time of the first inoculation did not exceed the average of the tumors employed for implantation at this period of the study, and yet 49 per cent. of successful inoculations were secured. And while the tumor used to inoculate the negative animals at the time of the second inoculations showed among the controls a lower virulence or a lessened capacity for growth than the tumor used for the first reinoculation, yet a further 14.2 per cent. of successful implantations were secured.

The case is not so clear as regards the natural refractory state's being increased by a first unsuccessful inoculation. Table II is suggestive of such an effect as regards the small number of successful reinoculations in the first thirty-day period following the unsuccessful implantation, as compared with the greater number of successful inoculations at later periods. But the figures given are indications merely, and do not serve to establish the point.

SECONDARY AND TERTIARY IMPLANTATIONS.

Ehrlich[4] has pointed out that in the case of actively growing mouse tumors, secondary implantations undertaken at a time at which the first tumor is growing rapidly usually fail to grow. Without discussing the theoretical views which he has offered to explain this observation, we wish to report the results of secondary and tertiary implantations carried out with this slow growing rat tumor. As has already been pointed out, this tumor exhibits in a high degree the property of metastasising in nearby and distant parts of the body, from which it is concluded that the effect on the organism of a growing tumor was not such as to interfere with or inhibit the growth of a secondary tumor. Table IV exhibits the results of the reinoculations, and serves to bring out several points of considerable interest.

TABLE IV.

Reinoculation of Rats with Growing Tumors.

Age of primary tumor at time of reinoculation.	Average size of primary tumor in cm.	Number reinoculated.	Number of primary tumors continuing to grow after reinoculation.	Number with growing tumors developing secondary tumors.	Number of primary tumors not growing or retrogressing.	Number with stationary or retrogressing tumors developing secondary tumors.	Number of secondary tumors retrogressing.
First 30 days	0.7	63	36	36	27	1	2
30 to 60 days	1.1	53	43	23	10	0	0
60 to 90 days	1.3	83	78	62	5	0	0
90 to 120 days	1.2	15	11	9	4	0	0
120 to 150 days	2.5	5	5	2	0	0	0
		Number of rats inoculated.	Number developing tumors.	Number in which tumors disappeared.			
Control		80	69	21			

It shows, in the first place, that a growing tumor does not prevent the successful implantation of a second tumor of the same kind, but it also indicates that the secondary implantations are likely to yield a smaller number of growing tumors, the susceptibility of the rats inoculated having been established, than the primary inoculation. The influence of the time element during which the first tumor has

[4] Ehrlich, *Arb. a. d. k. Inst. f. exper. Therapie,* 1906, No. 1, 77.

been growing in the body appears to be of some importance in determining the result, but the factor that exercises a definite and determining influence is not the original susceptibility of the animals to inoculation, but the state of the primary tumor with reference to its later history.   So long as the primary tumor is itself expanding the secondary inoculations are readily accomplished, but when the primary tumor has become stationary in size, or has begun to undergo retrogression, the secondary implantation usually fails.   In only one instance among 46 cases did a rat in which the primary tumor was being absorbed develop a secondary tumor.   In view of the fact that rats showing retrogressing tumors do not develop secondary tumors, while those with growing tumors tend to develop them, it would be expected that few or none of the secondary tumors should later suffer retrogression.   Table IV shows that in only two rats was there a disappearance of the secondary growths.

The influence of the period of growth of the primary tumor on the result of the secondary implantations is shown by the fact that in the first thirty-day period all the rats in which the primary tumor continued to grow developed secondary tumors, and that some rats with growing tumors in the later periods did not develop secondary tumors.[5]

Still another test of the reinoculability of rats with growing tumors was carried out with 29 animals in which two tumors were growing at the time of the third inoculation.   This implantation was made during the period of from 30 to 60 days of the duration of the two growing tumors, with the result that of the 29 rats inoculated 23 developed tertiary tumors.   It follows from these observations that with the rat tumor with which these tests are made reinoculation is possible in all or nearly all the animals, provided the tumors which originally developed from the first implantation are still growing, and have not either become stationary, or begun to undergo retrogression.

[5] Recently Gay has attempted to show that rats inoculated with this tumor are subject to reinoculation only after the period of metastasis has been reached, and that at an earlier period the result of a secondary inoculation is to cause rather absorption of the original tumor than the successful implantation of the second.   These statements are in direct conflict with the observations represented by Table IV.

PROMOTING INFLUENCE OF TUMOR EMULSION.

In the course of experiments performed with a number of organic and other substances to be related, the influence on the growth of the tumors of an emulsion of the tumor substance itself was studied. Previous experiments had shown that fine emulsions of the tumor

TABLE V.

Effect on Tumor Growth of Heated and Unheated Tumor Emulsion.

Mode of treatment.	Treated with emulsion.			Control.	
	Number inoculated.	Number and per cent. developing tumors.	Number and per cent. in which tumors disappeared.	Per cent. developing tumors.	Per cent. in which tumors disappeared.
Unheated emulsion one day and tumor next day	8	7 (87.5)	3 (42.7)	100	70
Heated emulsion one day and tumor next day	10	10 (100)	5 (50)	20	50
Tumor one day and heated emulsion next day	9	9 (100)	6 (66)	100	70
Unheated emulsion subcutaneously and tumor 10 days after	10	6 (66)	6 (100)	80	75
Unheated emulsion intraperitoneally and tumor 10 days after	10	7 (70)	6 (85)	80	75
Heated emulsion subcutaneously and tumor 10 days after	28	28 (100)	4 (14)	82.5	36
Heated emulsion twice at 10-day intervals and tumor 10 days after second injection	10	10 (100)	1 (10)	70	56.2
Heated emulsion three times at 10-day intervals and tumor 10 days after third injection	10	10 (100)	0	87	83.6
Heated emulsion and tumor 20 days after	10	10 (100)	2 (20)	70	56.2
Heated emulsion and tumor 30 days after	10	10 (100)	4 (40)	87.5	83.6
Heated emulsion twice at 10-day intervals and tumor 20 days after second injection	10	8 (80)	0	87.5	83.6
Heated emulsion three times at 10-day intervals and tumor 30 days after third injection	9	9 (100)	1 (11)	90	55.5

in salt solution did not give rise to tumor formation, so that there was little or no risk of having tumors develop from the injected emulsion. The emulsion which was made of a uniform milky appearance was divided into two portions, one being subjected to

heat in a water-bath, at a temperature of 55° C. for thirty minutes before the injection, and the other not being further treated. A standard suspension of lecithin was used as control. Injections of one cubic centimeter of the emulsion were made into the peritoneal cavity and the tumor material used was of the type of growth not definitely adeno-carcinomatous. The experiments have not been repeated since the tumor assumed the latter form. At a later period, and, therefore, subsequent to the injection of the emulsion, tumor fragments were inoculated beneath the skin, and the results observed, as recorded in Table V.

As the table shows, the effects of the injections of the emulsion are considerable. They will now be taken up separately for consideration.

The effects of the unheated and of the heated emulsions begin to be displayed as early as twenty-four hours after their injection, and in about the same degree. Thus the number of positive inoculations is the same in both series, and the number of retrogressions is also about the same. As compared with the control series, the effect of the unheated emulsion is seen in the smaller number of disappearing tumors, and of the heated emulsion in an increasing number of positive implantations. It chanced that the tumor chosen for the experiments with the heated emulsion gave in the controls a low percentage of positive implantations, but as the same tumor was inoculated into the animals previously having had injections of the heated emulsion, the contrast is very marked. On the other hand, the injection of the heated emulsion on the day following the implantation of the tumor morsels is followed by little or no special influence on the number of retrogressing tumors, since they were about the same as in the control series.

The next set of experiments yielded much more striking results. Unheated emulsion was injected subcutaneously and intra-peritoneally, and heated emulsion subcutaneously ten days before the tumor implantations. The rats which had received the unheated emulsion behaved very much as did the controls, since the number of positive inoculations and of retrogressions was about average, while the rats receiving the heated emulsion gave 100 per cent. of positive implantations, and a small number, 14 per cent., of

retrogressions, as against 82.5 per cent. of positive implantations and 36 per cent. of retrogressions in the control series. It appears, then, that rats treated ten days in advance with a heated tumor emulsion show increased susceptibility to the tumor implantations and a diminished tendency to tumor retrogressions, as compared with untreated rats or rats treated with unheated emulsion. Indeed, the unheated emulsion proved not to exhibit any predisposing effect whatever on the growth or the persistence of the tumors.

Another series of experiments was performed with the heated emulsion. The experiments were so arranged that the injections were made two or three times at ten day intervals, and the tumor implantations were conducted at ten, twenty, and thirty day intervals after the second and third injections. These experiments will show the promoting influence of the heated emulsion on the growth and endurance of the tumors. We possess no knowledge of the nature of the constituent of the heated emulsion upon which depends the effect described, or of the mechanism of the promoting influence. In view of facts already established for mice tumors, that blood corpuscles and several kinds of tissues of the same animal species exercise, when injected in advance of the tumor implantations, a restraining influence on the tumor growth, the absence of such a restraining influence following the development of the unheated emulsion of tumor and the development of the promoting qualities following the injection of the heated emulsion are points worthy of special attention. It remains to be ascertained whether this effect is peculiar or limited to this rat tumor, or to a small number of transplantable tumors, or whether it is applicable equally or in some degree to transplantable tumors in general. We are engaged at present in elucidating this point.

TABLE VI.

Summary of Effect on Growth and Persistence of Tumor of Heated Emulsion.

Series.	Total number of rats treated.	Number and per cent. developing tumors.	Number and per cent. in which tumors disappeared.
Control	28	26 (92.8)	19 (73)
Unheated emulsion	28	20 (74.2)	15 (75)
Heated emulsion	106	104 (98.1)	23 (22)

We have summarized in Table VI the effects of the heated emulsion on the growth and persistence of the tumor.

Thus it will be seen that among 28 control rats 26 developed tumors, equalling 92.8 per cent., of which 19, or 73 per cent., later underwent retrogression. Among 28 rats treated with unheated emulsion, 20, or 74.2 per cent., developed tumors, of which 15, or 75 per cent., underwent retrogression. These figures are to be contrasted with the next, in which of 106 rats treated with the heated emulsion, 104, or 98.1 per cent., developed tumors, of which 23, or 22 per cent., later were absorbed.

The fact should be mentioned that when a very large number of animals were to be inoculated a large tumor was always selected, so as to insure uniform results. As the large tumors are more advanced in degree than the small ones, they tend to give a lower percentage of successful implantations and a higher percentage of retrogressions. In the present case these points emphasize the effects of the heated emulsion.

EFFECT OF HEATED EMULSION ON IMMUNE RATS.

As has been already pointed out, rats which have recovered spontaneously from the tumors possess an increased degree of resistance to reimplantation of tumor fragments. In view of the power of the heated emulsion to overcome the natural resistance to the implantation of the tumor, it became desirable to ascertain whether this induced or increased resistance could also be set aside by the emulsion. The experiments made to test this point were carried out on two small series of rats which had recovered from tumors that had developed in animals previously receiving the heated emulsion.

In the first series of eight rats, the tumors had entirely disappeared for a period of from 30 to 90 days, and they received no new injection of the heated emulsion. Three of the eight rats developed tumors from the new implantations, of which one tumor subsequently disappeared. The second series of nine rats had been free of tumor for a period of from 30 to 60 days. The heated emulsion was again injected ten days prior to the second tumor implantations. Four of these developed tumors, of which two subsequently disappeared. The control rats for these series gave 100 per cent. of

successful inoculations and no retrogressions, indicating that the tumor used for implantation was of maximum virulence. There is, therefore, no evidence, so far as this experiment goes, of any influence of the emulsion in promoting successful implantation in these rats.

The experiment was now extended to rats which had not been previously injected with the heated emulsion, but which had recovered spontaneously from tumors. They received the heated emulsion in the usual way, and afterwards implantation of tumor fragments. Two series of control animals were employed: one consisting of animals not previously inoculated, to establish the virulence of the tumor, and the other " immune " rats, so-called, in which no emulsion had been introduced. Table VII summarizes this experiment, from which it will be seen that of ten rats in which tumors had disappeared, from 30 to 120 days previously, one only developed a tumor, and this subsequently disappeared; whereas the ten control animals, which had not been previously inoculated, all developed

TABLE VII.

Effect of Heated Emulsion on Immune Rats.   No Emulsion   (control.)

Time period since disappearance of tumor.	Number inoculated.	Number developing tumors.	Number in which tumors disappeared.
30 to 120 days	10	1	1
Control	10	10	0
Emulsion on Same Day as Tumor.			
30 to 90 days	10	3	0
Emulsion Ten Days Before Tumor.			
30 to 120 days	10	5	0

tumors, of which none disappeared. These results are to be compared with those obtained in animals in which the injection of the emulsion and the implantation of the tumor were made on the same day, and the emulsion injected one day and the tumor implanted ten days subsequently, as are shown in the lower part of the same table.

Thus, of ten rats which had recovered from 30 to 90 days previously, and which received the emulsion and the tumor on the same

day, three developed tumors; whereas of the ten which had recovered from 30 to 120 days previously, and which received the tumor ten days after the heated emulsion, five developed tumors, in both cases there being no retrogressions.

The experiments are quite suggestive and indicate that the heated emulsion can overcome in some degree the induced resistance or immunity produced by the absorption of the tumor, and also that while this influence exerted by the emulsion is greater in rats which had not previously received the emulsion, it may still be exerted upon animals possessing the increased resistance given by recovery from the tumor following the emulsion. Moreover, the effect begins to be apparent on the same day as the injection of the emulsion, although it is more pronounced ten days afterwards.

EFFECTS OF THE HEATED EMULSION ON RATS WITH DISAPPEARING TUMORS.

Rats with growing tumors may be inoculated a second and a third time with tumor grafts. Rats recovering spontaneously from tumors acquire an increased resistance to tumor implantation. Hence, recovery from the tumor is attended by a rise in resistance in the organism associated with the absorption of the tumor, or to local conditions that affect the nutrition of the growth. The interesting question, therefore, arises as to whether this state of increased resistance which prevails during the retrogression is general or local, and whether it is capable of preventing the development of a secondary graft and can be overcome by the promoting influence on the tumor growth exercised by the heated emulsion. The incidental question was also put, namely, whether a secondary graft in an animal with a disappearing tumor can affect that tumor in such a way as to bring about a renewal of its growth. Table VIII supplies the answer to these questions as far as they have been worked out.

By a system of measurement we were able to ascertain fairly precisely the period at which growth ceased in the tumors and retrogression set in. In this experiment particular attention was paid to these two points. The results seem to indicate that the heated emulsion may overcome in some degree the condition of the organism

that operates to bring about tumor absorption. This indication, which we wish to state guardedly, is based on the observation that of the ten rats with retrogressing tumors receiving the emulsion, in only two did the tumors progress to complete disappearance, which is in contrast to the usual observation. Generally speaking,

TABLE VIII.

Secondary Inoculation of Rats with Disappearing Tumors. No Treatment.

Time period since retrogression began.	Number of rats in series.	Number from which the tumor disappeared.	Number of these rats developing new tumors.	Number in which tumors renewed growth.	Number of these rats developing new tumors.	Number in which primary tumors remained stationary.	Number of these rats developing tumors.
30 to 90 days	10	5	none	3	3	2	2
Control	10	All developed tumors					

Emulsion Ten Days Before Tumor.

30 to 90 days	10	3	1	4	4	2	1
Control	10	All developed tumors					

when retrogression has once begun, it proceeds to complete disappearance, provided the animals survive the necessary period. Moreover, while in the control series no new implantations developed in animals in which the primary tumors disappeared, yet one of three such animals of the series receiving the emulsion developed a secondary tumor.

A further result of this experiment was the successful implantation of new tumor grafts upon rats with retrogressing tumors. In view of this result, it may be considered as doubtful whether the state of the organism as a whole influences at all times the local tumors in respect to growth and recession, and whether local activities, and particularly the nutritive conditions, may not well play at times the determining part. It has been observed by Velich, Loeb[6] and others that the excision of a part of a stationary or retrogressing tumor nodule may be followed by renewed activity, which is explicable most readily on the supposition that the limitation of growth is affected by local conditions. But a somewhat more significant point developed by this experiment is the awakening, as it were, of

[6] Loeb, *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1902, clxvii, 175.

growth in the receding tumors by means of a secondary inoculation. Thus in seven of twenty rats showing receding tumors, a new growth began, following and apparently as the result of the reinoculation of the secondary graft.

EFFECTS OF ORGANIC SUBSTANCES ON TUMOR GROWTH.

The question which next arises concerns the element of the tumor emulsion upon which its promoting influence depends. We have already stated that we have not been able to determine this substance. We have, however, endeavored to ascertain whether the substance resides in the fluid serum or lymph contained within the emulsified tumor, or within the cells themselves. We have approached this question indirectly by testing the effect of the blood serum of the rat itself. We have, moreover, had in mind in the planning of these experiments the statement of Gaylord and Clowes,[7] that the blood serum of mice which have recovered spontaneously from implanted tumors exercises an immunizing or restraining effect on the development of implanted grafts of mouse tumors, and these effects are not exhibited by the serum of normal rats. It is true that Michaelis,[8] Ehrlich[9] and others have not confirmed this experiment, but since it lay in our way, we have not failed to make certain observations to cover this point.

For the purpose of this experiment the blood serum from several different series of rats was employed. Thus, the effects of the serum obtained from normal rats was compared with those of the serum obtained from rats which had failed to develop tumors on inoculation, and, hence, were designated "negative"; from rats which had recovered spontaneously from growing tumors, and designated "immune"; and finally, from rats possessing growing tumors at the time the serum was collected, and designated "positive." This series of experiments was controlled with bouillon, Ringer's solution, normal horse serum, and heated and unheated emulsion of the tumor itself. The procedures were in different experiments somewhat varied, so that this serum and the other sub-

[7] Gaylord and Clowes, *Johns Hopkins Hosp. Bull.*, 1905, xvi, 130.

[8] Michaelis, *Zeit. f. Krebsforsch.*, 1906, iv, 1.

[9] Ehrlich, *Arb. a. d. k. Inst. f. exper. Therapie*, 1906, No. 1, 77.

stances were given sometimes before and sometimes after the tumor implantations, as is indicated in the tables.

Table IX summarizes the experiment made with these substances which were given on the day preceding that on which the tumor implantations were made.

TABLE IX.

Effect on Tumor Implantations of Blood Serum, etc., Given Preceding Day.

Substance injected.	Number of rats inoculated with tumor.	Number and per cent. developing tumors.	Number and per cent. in which tumors disappeared.
None—control	10	10 (100)	3 (30)
Bouillon—control	10	5 (50)	4 (80)
Normal rat serum	10	8 (80)	7 (87.5)
" Positive " rat serum	5	5 (100)	3 (60)
" Negative " rat serum	6	5 (83)	2 (40)
" Immune " rat serum	4	4 (100)	1 (25)

The summary is instructive, since it shows, apparently, that the bouillon exercises a certain restraining effect, and that next to the bouillon the normal rat serum is the most influential substance. On the other hand, the serum from the so-called " positive " rats did not restrain the original taking of the grafts, while of the series of five animals employed three subsequently suffered retrogression of the tumors, which is a much higher percentage than the retrogressions suffered by the control series. The serum from the rats designated "negative" and "immune" affected the original implantations and retrogressions practically not at all as compared with the control series.

TABLE X.

Effect on Tumor Implantations of Blood Serum, etc., Given Following Day.

Substance injected.	Number of rats inoculated with tumor.	Number and per cent. developing tumors.	Number and per cent. in which tumors disappeared
None—Control	10	2 (20)	1 (50)
Bouillon—Control	10	3 (30)	2 (66)
Horse serum—Control	10	2 (20)	1 (50)
Ringer's solution—Control	10	6 (60)	5 (83)
Normal rat serum	10	6 (60)	5 (83)
" Positive " rat serum	4[10]	3 (75)	3 (100)
" Negative " rat serum	10	4 (40)	2 (50)
" Immune " rat serum	10	4 (40)	3 (75)
Heated emulsion	10	10 (100)	5 (50)

[10] Six of the ten rats inoculated died soon after the inoculation.

Table X exhibits the effects of the injections of the various substances carried out on the day following the tumor implantations.

It chanced that in this experiment the implanted tumor was of poor growing property. In some respects this was an advantage, inasmuch as it allowed for the play and exhibition of influences that are not so easily detectable where the power of growth is maximum. It is clear from the experiment that the rat sera tend rather to promote than to inhibit the success of the implantations, but what is particularly striking in this experiment is the promoting influence on the original tumor graft of the heated emulsion.

Table XI exhibits the influence of these substances when given ten days before the tumor implantation.

TABLE XI.

Effect on Tumor Implantation of Blood Serum, etc., Given Ten Days Before.

Substance injected.	Number of rats inoculated with tumor.	Number and per cent. developing tumors.	Per cent. in which tumors disappeared.
None—Control	10	8 (80)	75
Bouillon—Control	10	7 (70)	71
Horse serum—Control	10	6 (60)	33
Ringer's solution—Control	10	7 (70)	71
Normal rat serum	10	9 (90)	33
" Positive " rat serum	10	9 (90)	33
" Negative " rat serum	10	8 (80) ·	62
" Immune " rat serum	10	9 (90)	33
Unheated emulsion, subcutaneously	10	6 (60)	100
Unheated emulsion, intraperitoneally	10	7 (70)	85.6
Heated emulsion intraperitoneally	9	9 (100)	22

It supports the previous observations in indicating that the nature of the rat serum plays no part in determining the result of the implantations, and that of all the substances employed the heated emulsion alone produces a marked and undeniable influence on the growth and persistence of the grafts.

The several tables bring out in spite of certain irregularities the important data sought by the experiments. It is perhaps possible to inhibit in some degree tumor development by injecting into the body a short time before the tumor fragments are implanted certain relatively indifferent fluids, of which bouillon seems to be as active as any. On the other hand, the injection at any period, as regards the tumor implantations, of the serum of the blood of the rat.

whether obtained from normal animals, animals carrying tumors, failing to develop tumors, or recovering from them, entirely fails to produce any effect either in the direction of restraining or of promoting growth. It is, therefore, safe to conclude that the property affording resistance or relative immunity to certain rats to the primary inoculation, or developed through spontaneous recovery from tumors once successfully implanted, does not reside in the serum of the blood of these animals.

EFFECT OF BLOOD CORPUSCLES AND OTHER TISSUES ON TUMOR GROWTH.

Bashford[11] observed that the injection of the blood of the mouse exercised a restraining influence on the development of mouse tumors. Schoene[12] observed that the injections of suspensions of mouse embryos produced a similar effect, and Michaelis[13] and Borrel[14] observed that the injections of suspensions of liver and spleen acted apparently in the same manner. The activity of the blood in this respect was shown by Bashford[11] to depend on the corpuscles, and not to reside in the serum, and it may be assumed that in the other cases mentioned it is also the cellular elements and not the fluids upon which the activities depend. The experiments to be related refer to the effects of blood corpuscles separated from

TABLE XII.

Effect on Tumor Growth of Blood Corpuscles Given on Preceding Day.

Substance injected.	Number of rats inoculated with tumor.	Number and per cent. developing tumors.	Number and per cent. in which tumors disappeared
None—control	10	10 (100)	7 (70)
Bouillon—control	10	6 (60)	3 (50)
Ringer's solution—control	10	9 (90)	3 (33)
Normal rat serum	9	8 (88)	1 (12.5)
Washed rat corpuscles	10	9 (90)	4 (66.6)
Unheated emulsion	8	7 (87.5)	3 (42.8)
Heated emulsion	9	9 (100)	6 (66.6)

[11] Bashford, *Annual Report of the Imperial Cancer Research Fund*, 1906, iv, 5.
[12] Schoene, *Münchener med. Woch.*, 1907, liii, 2517.
[13] Michaelis, *Zeit. f. Krebsforsch.*, 1906, iv, 1; *Deut. med. Woch.*, 1907, xxxiii, 1826.
[14] Borrel, *Bull. de l'Inst. Pasteur*, 1907, v, 605.

the serum, washed, suspended in salt solution, and injected into the body, on the tumor implantations. The blood corpuscles and the substances used for controls were injected on the day preceding the implantation of the tumor, or ten or twenty days preceding that operation.

Table XII exhibits the effects produced on the tumor growth by an injection of corpuscles made on the day preceding the tumor grafts.

At the end of this brief period no effect has been produced, since the number of successful inoculations is about the same as those of the controls, which indeed is maximum, while the number of retrogressions is about the equivalent of the controls, indicating that no marked inhibition of growth through the development of increased resistance has been produced. On the other hand, the effect of the injections of blood corpuscles ten days before the tumor implantation is unmistakable, since, as Table XIII shows, not only is the number of successful inoculations greatly reduced, as compared with the control, but all of the tumors which originally developed underwent subsequent retrogression and disappeared.

TABLE XIII.

Effect of Blood Corpuscles Given Ten Days Before Tumor Implantation.

Substance injected.	Number of rats inoculated with tumor.	Number and per cent. developing tumors.	Number and per cent. in which tumors disappeared.
None—control	10	10 (100)	0
Normal rat serum	10	8 (80)	4 (50)
Washed corpuscles	10	4 (40)	4 (100)

The effect of the blood corpuscles is still present as late as twenty days after their injection, as can be seen by reference to Table XIV.

TABLE XIV.

Effect of Blood Corpuscles Given Twenty Days Before Tumor Implantation.

Subtance injected.	Number of rats inoculated with tumor.	Number and per cent. developing tumors.	Number and per cent. in which tumors disappeared.
None—control	10	10 (100)	3 (33)
Normal rat serum	10	10 (100)	2 (20)
Washed corpuscles	10	7 (70)	5 (71.4)

Apparently the effect is disappearing, since the number of successful implantations has not only increased over those of the ten-day period, although still smaller than the controls, but the number of retrogressions has also diminished.

The effects, therefore, of the injection of washed corpuscles of the rat into the peritoneal cavity on the development of grafts of this rat tumor placed beneath the skin is shown by these experiments to be considerable. The effect is not developed within twenty-four hours of the injection of the corpuscles, at which time such inhibition as has been afforded by bouillon, for example, is at its height. But at the expiration of ten days the inhibitory effect of the washed corpuscles is very great, and this effect is still appreciable at the expiration of twenty days, but how much longer it persists we have not undertaken to ascertain. This is in conformity with Bashford's observations on mice, who found that the refractory condition produced by the injection was not so marked four days after the injection as at the expiration of ten days, and that it persisted for at least three weeks.

The final experiment of this series was made with emulsions of several organs in a manner similar to that employed with the blood corpuscles and also with the emulsions of the tumor. These suspensions were injected into the peritoneal cavity, and ten days later the implantations of the tumor fragments were made beneath the skin. Table XV summarizes the results of this experiment.

TABLE XV.

Effect on Tumor Growth of Organ Emulsions Given Ten Days Before.

Substance injected.	Number of rats inoculated with tumor.	Number and per cent. developing tumors.	Number and per cent. in which tumors disappeared.
None—control	10	8 (80)	7 (75)
Unheated liver	10	6 (60)	3 (50)
Heated liver	10	4 (40)	1 (25)
Unheated muscle	10	8 (80)	3 (37)
Heated muscle	10	8 (80)	1 (10)
Unheated spleen	10	8 (80)	3 (37)
Heated spleen	10	5 (50)	2 (40)
Unheated kidney	10	8 (80)	3 (37)
Heated kidney	10	8 (80)	4 (50)
Unheated testicle	10	8 (80)	2 (25)
Heated testicle	10	8 (80)	2 (25)
Unheated tumor	10	5 (50)	4 (80)
Heated tumor	10	10 (100)	8 (80)

It is obvious from examination of the table that emulsions of the organs produce no remarkable change in the organism of the rats, through which the development of the tumor grafts is seriously inhibited.   There are, indeed, four places in the table which indicate that inhibition of some degree had taken place.   These are in connection with the heated and unheated liver, the heated spleen, and the unheated tumor emulsion.   The greatest degree of inhibition observed was in connection with the heated liver, but as compared with the action of the blood corpuscles it may be said that the organs containing much blood tend to be more inhibitory than those which contain little blood, and no action would appear to be exerted by the cells peculiar to the organs.   Lastly, as far as the results obtained from the heated organic emulsions can be compared with those obtained from the heated tumor emulsions, they may be said to be of the reverse character.

EXPERIMENTS ON THE INFLUENCE OF RADIUM BROMIDE ON A CARCINOMATOUS TUMOR OF THE RAT.

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INTRODUCTION.

A considerable number of observations have already been published regarding the use of radiation by means of radium bromide in the treatment of malignant tumors. One of the earliest investigators of the subject was Exner,[1] who employed it in the treatment of epithelioma and sarcoma, and who was the first to report on the microscopic examination of tumors so treated. He noted particularly the overgrowth of connective tissue which preceded the disappearance of the tumor cells, and which corresponded in amount more or less with the reduction in size of the growth. The observations which followed Exner's publication were not always so favorable. The greater number of reports emphasized the beneficial effects of radium upon rodent ulcer, which unless it extends over a wide surface is said readily to improve and to disappear completely under the influence of the radiations. Those who have reported successful results are Lehmann,[2] Foreau de Courmelles,[3]

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[1] Exner, Ueber die Behandlung von Oesophaguskarzinom mit Radium-strahlen, *Wiener klin. Woch.*, 1904, xvii, 96; Ueber die Art der Rückbildung von Karzinommetastasen unter der Einwirkung der Radiumstrahlen, *ibid.*, p. 181; Ueber die bisherigen Dauerresultate nach Radiumbehandlung von Karzinomen, *Deutsche Zeit. f. Chirurgie*, 1904, lxxv, 379.

[2] Lehmann, Essai sur l'action thérapeutique du Radium, *Arch. gén. de méd.*, 1906, i, 1301.

[3] Foreau de Courmelles, Un peu d'histoire de la thérapeutique du radium, *Actualités médicales*, 1904, xvi, 84.

Czerney,[4] Braunstein,[5] McLeod,[6] Sichel,[7] McIntyre,[8] Davidson,[9] Scholz,[10] Schwarz,[11] Abbe,[12] and others.   There is difference of opinion as regards its merits in the treatment of epitheliomata proper.   Undoubtedly a considerable number of growths thus treated begin to improve almost immediately, and gradually entirely disappear, while others, apparently similar, continue on the contrary to extend in spite of the treatment.   Thus Lehmann, Abbe, Czerney and Scholz report cures in many cases, and Foreau de Courmelles speaks of improvement through its use, while others report either negative or very transitory beneficial effects.   The results with the more malignant forms of tumors have been almost uniformly unpromising.   In the case of carcinoma of the esophagus producing constriction, it has been possible through the use of a specially constructed apparatus to produce amelioration of some of the most distressing symptoms which result from the obstruction (Exner and Einhorn),[13] but in no case was a cure effected.   Abbe states that he observed carcinomata to disappear under the influence of radium, but he also states that there was a marked tendency towards recurrence.

The results obtained through the employment of radium in transplantable mouse tumors have been more gratifying.   Apolant[14]

[4] Czerney, *Zeit. f. Krebsforsch.,* 1907, v, 27.

[5] Braunstein, Ueber die Wirkung der Radium-emanation auf bösartige Tumoren, *Therapie d. Gegenwart,* 1904, xlv, 412.

[6] McLeod, Further Observations on the Therapeutic Value of Radium, *Brit. Med. Jour.,* 1904, i, 1366.   [7] Sichel, *Brit. Med. Jour.,* 1904, i, 182.

[8] McIntyre, Radium and Its Therapeutic Effects, *Brit. Med. Jour.,* 1903, ii, 1524.

[9] Davidson, Radium Bromide, *Brit. Med. Jour.,* 1904, i, 181.

[10] Scholz, Ueber die physiologische Wirkung der Radium-strahlen und ihre therapeutische Verwendung, *Deutsche med. Woch.,* 1904, xxx, 94.

[11] Schwarz, Ueber die Wirkung der Radium-strahlen, *Pflügers Archiv,* 1903, c, 532.

[12] Abbe, The Subtle Power of Radium, *Med. Record,* 1904, lxvi, 321; The Specific Action of Radium as a Unique Force in Therapeutics, ibid., 1907, lxxii, 583.   Radium in Surgery, *Jour. of the American Med. Assn.,* 1906, xlvii, 183.

[13] Einhorn, Radium Treatment of Cancer of the Esophagus, *Jour. of the American Med. Assn.,* 1905, xiv, 8; Radium Receptacles for the Stomach, Esophagus, and Intestines, *Med. Record,* 1904, lxv, 399.

[14] Apolant, Ueber die Einwirkung der Radium-strahlen auf das Karzinom der Maüse, *Deutsche med. Woch.,* 1904, xxx, 454; Ueber die Rückbildung der Maüsekarzinome unter dem Einfluss der Radium-strahlen, *Deutsche med. Woch.,* 1904, xxx, 1126.

caused the inoculated carcinoma in mice to undergo retrogression in all the animals exposed to the radiations, and a large percentage subsequently entirely disappeared. He states as a result of the microscopical examination of tumors exposed to radium that no relation existed between the amount of radiation and the degree of change induced, and he advanced the theory that the primary action of the rays was on the tumor cells, and that there resulted from this a secondary stimulus which led to an overgrowth of the connective tissue that was characteristic of the disappearing tumors. Bashford,[15] who used the Jensen tumor, corroborated Apolant's results, but he attributed the injurious action of the radium to a variety of factors.

EXPERIMENTAL OBSERVATIONS.

The radium used in the experiments to be related was kindly supplied by Mr. Hugo Lieber, of New York, and later by Dr. Robert Abbe. The specimens supplied by Mr. Lieber were contained in hard rubber capsules covered with a mica plate, and in the form of celluloid coatings. Of the former there were three grades: (1) 10 milligrams of so-called 1,000,000 activity; (2) 25 milligrams of so-called 10,000 activity; and (3) 25 milligrams of so-called 1,000 activity. The celluloid disks were coated with gelatin containing radium of 10,000 and 25,000 so-called activities.

These latter coatings are said to possess the advantage of yielding the maximal action of all the rays emitted, since they can be brought into immediate contact with the skin without any intervening substance which may absorb the less penetrating rays. As far as could be ascertained with the apparatus at hand, and through the use of the electroscope, no loss in activity took place from the coatings after they had been in contact with the underlying tissues, although the tissues themselves were proven to have become radio-active. The coatings were bound by means of plaster strips to the skin covering the tumors, and the capsules were fitted into small felt pads possessing circular openings and were also bound in the same manner

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[15] Bashford, Action of Radium on Transplanted Mouse Tumors and Its Relation to the Spontaneous Arrest of Their Growth, *Scientific Report of the Imperial Cancer Research Fund*, 1905, No. 2, Part II, p. 56.

to the skin.   The period of radiation ranged from five minutes to six hours, and there was also considerable variation in frequency of treatment.

The rat tumors subjected to radiation belonged to the transplantation generations between the ninth and the seventeenth, inclusive, and corresponded to the stage of simple carcinoma and adeno-carcinoma.   The method was to select a number of inoculated animals for radiation, and to retain a similar number of animals in a corresponding condition, as controls.   The size of the tumors at the beginning of the radiation varied from 0.5 to 2 centimeters in diameter.

It is noteworthy that the effects of the first radiations were very severe, not on the tumor so much as on the general condition of the animals.   That is to say, all the rats subjected to radiation in the first few days of the experiment died.   This result was attributed to the fact that the radium had been lying undisturbed in a leaden box for a period of some months, and there had, therefore, been an accumulation of rays and possibly the alpha rays especially, which proved injurious in the manner indicated.   A similarly unfortunate result followed the first application of the celluloid coatings, which had also remained unused for several months.   Later on, after the treatment had been inaugurated, no such deleterious effects were encountered.

In all but three of the animals submitted to radiation with the Lieber specimens the nodules continued to grow.   Complete disappearance occurred in three instances after radiation with the 1,000,000 specimen.   These animals showed on subsequent postmortem examination normal organs.   At first sight it might appear that the disappearance of these tumors was referable to the radiation, but in view of the considerable tendency to spontaneous retrogression exhibited by this tumor this assumption is not wholly justified. Indeed, the decision in this case would have to be made, not from the number of retrogressions occurring, but rather from the number of tumors which continued to extend.   Viewed in this light, the inevitable decision would be that the radiations had not produced the disappearance of the tumors.

There is one cause of temporary diminution in the size of the

tumors subjected to radiation that should be mentioned. When ulceration takes place there occurs frequently an apparent diminution in the size of the nodules, but this is produced through the loss of tissue from sloughing and subsequent collapse of the peripheral thin shell remaining. The ulceration tends to occur earlier in the radiated than in the non-radiated nodules, probably because of the injury to the skin produced by the radiation. Now the tumors which have undergone this early ulceration, far from being retarded in their growth, continue to extend actively at the borders and ultimately to produce metastases and to cause death.

A series of experiments was made on animals which had received the tumor inoculations on the two sides of the body. The purpose of the experiments was to ascertain if the radium when applied to the tumor on one side would exert any influence on the growth of the tumor on the opposite side of the body, or if the structure of the tumors on the two sides would exhibit any marked differences. The results of the experiments indicated that no special influence was exerted by the radiation either on the tumor immediately treated or on the one on the opposite side. The relative rates of growth showed nothing that the controls did not also show, and the microscopical characters of the two sets of tumors were also in agreement.

At the conclusion of the first series of experiments, which were quite negative in result, we secured through the kindness of Dr. Abbe the use of a preparation of radium much stronger than any which we had previously employed. This specimen consisted of a hard rubber capsule as described, containing ten milligrams of 1,800,000 radio-active radium bromide. This constitutes what Dr. Abbe calls a standard cell. Through its use and his aid by the use of his photographic method we were able to standardize the various preparations loaned us by Mr. Lieber. Thus the specimen containing ten milligrams of 1,000,000 radium was shown to be $\frac{1}{8}$ of the standard strength, and the twenty-five milligrams of 10,000, $\frac{1}{25}$ of of the standard strength. The coatings of 10,000 and 25,000 radio-activity were respectively about $\frac{1}{500}$ and $\frac{1}{300}$ of the standard cell. To obtain the theoretical effects of the standard cell it would therefore be necessary to continue the radiations with the weaker

preparations as many times longer as they are weaker than the standard cell.

Twenty-four rats possessing tumors were subjected to radiation with Dr. Abbe's standard cell, of which nineteen were radiated for one hour, two for two hours, one for three hours, one for one hour and twenty-five minutes, and one for one hour and twelve minutes. The size of the tumors in this series of rats at the time of the radiation ranged from 0.4 by 0.6 centimeters to 1.0 by 1.6 centimeters.

The first noticeable effect of the radiations was the loss of hair over the radiated area, which was accompanied by a severe dermatitis, followed from ten days to two weeks later by ulceration in several nodules.   The area of necrosis and original ulceration corresponded exactly to the dimensions of the overlying exposed surface of the radium.   The ulcers, once formed, extended afterwards. In six of these twenty-four animals the tumors underwent shrinkage, and in four of these six, in which the tumors were much smaller, they completely disappeared.   To some of the animals which survived and in which retrogression did not take place, a second radiation of one hour was given a month later, but no influence was exerted on the progress of the tumors.   The results of this experiment must be interpreted in the manner of the last, that is to say, the retrogressions were not more numerous than often occur spontaneously, and the fact that so many of the tumors continued to extend in spite of the radiations indicates that this tumor when developing beneath the skin is not subject to marked inhibition of growth through the radiation.

In order to represent the experiments which were carried out, and the results obtained, the number of animals in each experiment, as well as the nature of the experiment, will be stated.

Nine rats were treated with the radium coatings.   The radiations were repeated weekly during the life of the animals.   In no case did the tumors disappear, and in every case but one in which there was survival beyond the first few days following the first radiation there was increase in size.   In one instance a partial shrinkage of the tumor took place, but complete disappearance never occurred.

Nineteen rats were submitted to radiation with the capsule of

10,000 radium bromide. The number of exposures varied from one to twenty-four. In two animals only did the tumors disappear. In all the others they continued to extend. Hence no effect on the development of the tumor was exerted by these radiations.

Eighteen rats were submitted to radiation with the 1,000,000 specimen of radium bromide. The radiations ranged from one to ninety in number, and from a few minutes to several hours in duration. In one instance only was there complete disappearance of the tumor, and in four instances was there practical arrest of the growth.

Twenty-four rats were submitted to radiation with the 1,800,000 specimen of radium bromide. In four rats the tumors completely disappeared and in two others there was arrest of development. In the others the tumors extended until the death of the animals.

Six rats in which tumors were growing on both sides of the body were submitted to radiation of the tumor on one side. The number of radiations varied from two to fifteen. The radium employed consisted of the 10,000 and 1,000,000 capsules. No appreciable healing effects were produced.

The almost uniformly negative results obtained with the radium in the treatment of the rats led to the testing of the radium preparations on certain mouse tumors. The Jensen tumor, which had previously been studied in this respect by Bashford, was first chosen. Eight mice, for which a number of control animals were kept, were submitted to radiation with the several Lieber preparations, for varying periods of time. In six of these eight the tumors completely disappeared in from two weeks to two months. The two tumors which had not completely disappeared were found on microscopical examination to be completely necrotic. No living cells were found in the tumor nodules proper. The next experiments with mice were carried out with animals in which the Ehrlich sarcoma was developing. The Abbe standard cell was employed, and the radiation continued for one hour. No appreciable effect was exerted upon this tumor, which continued to grow in all the animals as it did in the controls. On subjecting these tumors to microscopical examination, no differences in structure as compared with the unradiated specimens could be discovered.

CONCLUSIONS.

The carcinoma of the rat with which we have dealt, when developing in the form of nodules beneath the skin and near the surface, is not subject to inhibition of growth and such injury as tends to produce retrogression through the influence of radium emanations. White rats are, on the other hand, highly susceptible to the injurious effects of certain of the radium emanations, which may produce death within a short time following the exposure. The local injurious effects of the emanations, when producing no general disturbance in the condition of the animals, suffice often to cause falling out of the hair, the development of a severe dermatitis and ulceration of the skin and the tumors. The extension of the tumors at the edges of the ulcer is not materially restrained.

The Jensen mouse tumor has been found by us as by others to be readily subject to the injurious effects of the radium emanations, which act upon it in such a manner as to lead to its disappearance without causing appreciable injury to the general health of the mice in which the tumor is growing. On the other hand, the Ehrlich spindle-cell sarcoma of mice is not subject to the inhibiting influence of the radium emanations, but continues to grow without apparent diminution of energy, in a manner similar to the control animals not submitted to radiation.

SPONTANEOUS TUMORS OF THE MOUSE.

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PLATES XVII–XXVIII.

Since Hanau[1] first showed, with a carcinoma of the rat, the possibility of successful transplantation of a malignant tumor from one animal to another of the same species, numerous observations have been made along similar lines.[2] Morau[3] showed that the mouse was subject to transplantation of carcinomatous tumors, and Loeb[4] demonstrated that other carcinoma and sarcoma could be successfully transplanted in rats. Jensen,[5] and later Loeb, carried out careful histological examinations of fragments of transplantable mouse tumors, removed at varying periods following inoculation, and ascertained that the new development of the tissue took place entirely from the transplanted epithelial cells and not from the tissue of the host. Bashford[6] has not only confirmed these observations, but he has shown conclusively that the stroma of the tumors is the element that is derived from the host. In recent years an immense activity has developed in connection with the experimental study of transplantable tumors, and many facts of fundamental biological importance have been settled in the course of this experimental investigation. In the following paper no special reference will be

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[1] Hanau, A., *Fortschr. d. Med.*, 1889, vii, 321.

[2] It is historically of interest to allude to the unsuccessful attempt of Joseph Leidy to transplant a human mammary carcinoma into the frog, reported by him to the Academy of Natural Sciences of Philadelphia in 1851 (*Proc.*, v, p. 201). Four pieces of the tumor, each half an inch long by one-eighth of an inch broad and thick, were inserted beneath the integument of the back of a large frog.

[3] Morau, H., *Arch. de méd. exper. et d'anat. path.*, 1894, vi, 677.

[4] Loeb, L., *Jour. of Med. Research*, 1901, vi, 28.

[5] Jensen, C., *Cent. f. Bakt., Orig.*, 1903, xxxiv, 28, 122.

[6] Bashford, E., *Scientific Report of the Imperial Cancer Research Fund*, 1905, No. 2, 24.

made to the general lines of investigation being followed, but there will be described, as briefly as possible, the histological structure of and the results obtained from the transplantations into mice of a considerable number of tumors developing spontaneously in that class of animals. During the past three years there have come into our hands twenty-six mice showing spontaneous tumors. These twenty-six animals showed, according to conclusions based upon my study, forty-one primary tumors. In one mouse there was present what appeared to be either a general hyperplasia of lymphoid tissue, or else many metastases from a large superficial lympho-sarcoma, but, of course, this animal was taken as representing one tumor formation. However, ten of the mice, or 38.4 per cent. of the entire number, showed more than one tumor each. Three of these showed three tumors each, and seven, two tumors each.

If we compare our observations with others reported in the literature, we shall find that they agree generally with previous observations, although differing considerably in detail. Thus Apolant[7] reported that among 221 mice showing spontaneous tumors he found 276 primary tumors; that is, 12 per cent. of the mice mentioned possessed more than one tumor. Tyzzer[8] has also reported multiple primary tumors in mice, and Murray[9] mentions that of 119 mice with spontaneous tumors 142 tumors in all were detected. In other words, in Murray's series 15 per cent. of the animals possessed more than one tumor. Reporting further, he states that fourteen mice showed two tumors each; three, three tumors each; and in one animal the entire inguinal mamma had been transformed into a group of nodules. In six of the animals of our series[10] there were present large subcutaneous tumors, and in six, what were taken to be primary tumors of the lung. One mouse showed a subcutaneous sarcoma, a cyst-adenoma of the ovary, and an adenomatous tumor of the lung; another mouse showed a superficial

[7] Apolant, A., *Arb. a. d. k. Inst. f. exper. Therapie,* Frankfurt, 1906, No. 1, 11.

[8] Tyzzer, E., *Fourth Report of the Caroline Brewer Croft Fund Cancer Commission,* 1907, 27.

[9] Murray, *Scientific Report of the Imperial Cancer Research Fund,* 1908, No. 3, 69.

[10] Jobling, *Proc. of the Soc. for Exper. Biol. and Med.,* 1908, vi, 10.

sarcoma together with cyst-adenomata of both ovaries; and, finally, two mice each presented two subcutaneous tumors of different types.[11]

TRANSPLANTATION.

The voluminous literature which has already accumulated on the transplantation of mouse tumors contains many statements of successful and unsuccessful transplantation, but there are available relatively a small series of observations which enable us to determine the percentage of transplantable tumors. Ehrlich[12] reports upon 230 tumors, of which 94 were inoculated into other mice, and 11 per cent. of these proved to be transplantable. Tyzzer[13] reports four carcinomata of mice, two of which were successfully transplanted. Bashford[14] reports that 15 out of 32 sporadic tumors transplanted gave negative results. Of our series of 41 tumors, 26 were inoculated into other mice, and of these 20, or 77 per cent., developed. From these tumors 1,128 mice were inoculated, of which 855 survived more than two weeks. Of these, 104, or 12.1 per cent., developed tumors. Of these 104 grafts which began to grow, 36, or 34 per cent., later underwent retrogression. The number of tumors developing in the first generations varied from 2 to 46 per cent. and the period of incubation of the first tumors ranged from 30 to 180 days. In Ehrlich's series 1,504 mice were inoculated with material from 94 primary tumors, 41 of which, or 2.7 per cent., developed tumors. The percentage of tumors developing in the first generation of our different implantations ranged from 2 to 50 per cent. Bashford[15] transplanted 32 spontaneous tumors of the

[11] Tyzzer in a later article (*Boston Med. and Surg. Jour.*, 1909, clxi, 103) says: "Different types of tumors frequently occur in a single animal. Of the 49 animals of this series 11 presented primary tumors of two types, and 1 animal, primary tumors of four types. In the last there were a hypernephroma, a lymphosarcoma, a papillary cyst-adenoma of the lung, and an adeno-carcinoma of the ovary."

[12] Ehrlich, P., *Arb. a. d. k. Inst. f. exper. Therapie*, 1906, No. 1, 77.

[13] Tyzzer, E., *Fourth Report of the Caroline Brewer Croft Fund Cancer Commission*, 1907, 27.

[14] Bashford, *Scientific Report of the Imperial Cancer Research Fund*, 1908, No. 3, 313.

[15] Bashford, *Scientific Report of the Imperial Cancer Research Fund*, 1905, No. 2, Part II, 19 and 30.

mamma into 2,278 mice, which survived a sufficient time to permit of a final estimation, and 72 tumors developed. That is, one inoculation in 31.1 mice was successful, yielding 3.2 per cent. of successes.

<div align="center">LOCALITY.</div>

In describing the location of the spontaneous tumors of 221 mice, Apolant[16] states that 25 per cent. were situated on the side of the abdomen, 16 per cent. in the region of the vulva, and 15 per cent. between the sternum and the submaxillary glands. Tyzzer[17] reports 20 cases, in which 5, including one lympho-sarcoma, were located in the subcutaneous tissues of the abdomen, 12 were primary lung tumors, one was a lympho-sarcoma of the thorax, and two were adenomata of the kidneys.[18] Murray[19] does not describe accurately the frequency with which the tumors were found in different parts of the body, but judging from the diagram prepared by him and showing the positions of 142 primary tumors, the great majority developed on the side of the thorax and in the inguinal regions.

In our series[20] of 41 primary tumors, 9 appeared in the lungs, 3 in the ovaries, and 29 in the subcutaneous tissues. Of the last, 4 were located in the neck, 9 on the side of the thorax, 14 in the inguinal region, and 2 in the region of the vulva. This series does not include the mouse in which there were multiple growths composed of lymphoid tissue.

[16] Apolant, A., *Arb. a. d. k. Inst. f. exper. Therapie*, 1906, No. 1, 11.

[17] Tyzzer, E., *Fourth Report of the Caroline Brewer Croft Fund Cancer Commission*, 1907, 27.

[18] In a later paper (*op. cit.*) Tyzzer says: " I have now studied a series of 62 primary tumors in mice, and of these, 37, or about 60 per cent., originated in the lung. The next most frequent type of tumor in my series is the lympho-sarcoma, of which there were 10. Only 8 of the 62 tumors were situated externally and 6 of these were epithelial tumors. There occurred 4 tumors of the kidney, of which 2 were undoubtedly hypernephroma. In 2 mice ovarian tumors occurred and in 1 mouse, a sarcoma."

[19] Murray, *Scientific Report of the Imperial Cancer Research Fund*, 1908, No. 3, 69.

[20] Jobling, *Proc. of the Soc. for Exper. Biol. and Med.*, 1908, vi, 10.

METASTASIS.

Hanau[21] in his original article reported the existence of metatases in the lymphatic glands in the neighborhoood of the cancer of the rat. Similar metastases, either appearing with the primary tumor, or as a result of transplantations, have been described by Loeb,[22] Borrel,[23] Haaland,[24] Apolant,[25] Flexner and Jobling,[26] Michaelis,[27] and Tyzzer.[28] With few exceptions the metastases have been confined to the lungs. Apolant reports that of his series of 221 mice suffering from spontaneous tumors, metastases in the lungs were found five times, or in 2.2 per cent. of the animals. Murray[29] reports that of 68 mice with spontaneous tumors, 27 presented lung metastases, and 3, lymphatic gland metastases. In 26 mice of our series lung metastases were found in 5, or in about 20 per cent. of the animals, and no metastases were discovered in any other organ of the body.

MAMMARY TUMOR.

Of the 41 tumors, 29 developed in positions of the body corresponding with the distribution of the mammary glands, and these, with the exception of 5, all presented well defined adenomatous structure. We have concluded in conformity with the present view of different workers that these tumors originated from the mammary gland. Attempts have been made to classify these tumors according to types. Michaelis[30] distinguishes three different types, and Haaland,[31] four; but probably the most satisfactory classification at present is that offered by Apolant.[32]

[21] Hanau, A., *Fortschr. d. Med.*, 1889, vii, 321.

[22] Loeb, L., *American Jour. of the Med. Sciences*, 1903, cxxv, 243.

[23] Borrel, *Ann. de l'Inst. Pasteur*, 1903, xvii, 81.

[24] Haaland, *Ann. de l'Inst. Pasteur*, 1905, xix, 165.

[25] Apolant, *Arb. a. d. k. Inst. f. exper. Therapie*, 1906, No. 1, 11.

[26] Flexner and Jobling, *Jour. of the American Med. Assn.*, 1907, xlviii, 420.

[27] Michaelis, *Zeit. f. Krebsforsch.*, 1907, v, 189.

[28] Tyzzer, E., *Fourth Report of the Caroline Brewer Croft Fund Cancer Commission*, 1907, 27.

[29] Murray, *Scientific Report of the Imperial Cancer Research Fund*, 1908, No. 3, 69.

[30] Michaelis, *Zeit. f. Krebsforsch.*, 1906, iv, 1.

[31] Haaland, M., *Ann. de l'Inst. Pasteur*, 1905, xix, 165.

[32] Apolant, H., *Arb. a. d. k. Inst. f. exper. Therapie*, 1906, No. 1, 11.

He begins by referring to the difficulty of classifying these mouse tumors, and speaks of the similarity of their histological structure to thyroid tumors of man. He distinguishes two main groups, adenoma and carcinoma, and further divides the adenoma into simple adenoma, cyst-adenoma, hemorrhagic cyst-adenoma and papillary cyst-adenoma. The carcinoma he divides further into simple alveolar carcinoma, hemorrhagic cyst-carcinoma, and papillary carcinoma. We have followed Apolant's classification in describing the twenty-five tumors of our series, composed of epithelial cells and stroma, and arising in the mammary region. These twenty-five tumors were composed of ten hemorrhagic cyst-adenomata, twelve adeno-carcinomata, two cyst-adenomata, and one alveolar carcinoma. Before proceeding to a somewhat more detailed description, it should be stated that these tumors are not strictly homogeneous, but that almost every one shows heterogeneous areas of a type differing more or less from the one under which it was classified, so the most that can be said is that the dominant structure has been chosen for the purpose of classification.

ADENO-CARCINOMA.

By far the greater number of the tumors of mice thus far reported belong to this class. It is interesting to recall that Hanau's[33] original study was of a squamous cell carcinoma of the vulva of the rat, but the tumors of mice and rats described since that time have been with few exceptions adeno-carcinomata of the mammary gland. Of the twelve spontaneous tumors of this class coming into my hands, ten were used for transplantation, of which nine were successfully transplanted into other mice. Of 464 mice inoculated with these tumors, 407 survived two weeks or longer, of which 57, or 14 per cent., developed tumors. The average period of incubation for this series was 63 days. Among the ten animals whose tumors were transplanted, four showed lung metastases and four also exhibited primary tumors of the lung.

These tumors were, with one exception, in which there was ulceration of the skin, freely movable and apparently encapsulated. The tumors in two of the series showed areas of keratinization.

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[33] Hanau, *Fortschr. d. Med.*, 1889, vii, 321.

The keratin transformation of the epithelial cells of these tumors has long been known. It was present in Hanau's original carcinoma, and has been described by Borrel and Haaland,[34] Erdheim,[35] Haaland,[36] Bashford[37] and Lewin.[38] In Lewin's case, as in Hanau's, the condition was present in tumors of the rat, and in all the others, in tumors of mice. In Lewin's case the keratinization did not appear until the third generation, when it became diffuse. In only one of the two cases observed by us did the transplanted fragments develop, and it is interesting to record that the keratinization did not appear in the subsequent tumors.

Generally speaking, these tumors are solid in structure and grayish-white in color. They are composed of microscopical lobules filled with closely packed, regular acini, which often contain a material of homogeneous structure staining deeply in eosin. That these tumors are to a certain extent invasive is shown by the presence of striated muscle fibers in the main tumor. The stroma separating the lobules tends to be dense and thick, although cellular in places, while that separating the acini is delicate and sometimes difficult to make out. The acini are usually lined with a single layer of cells, cubical in shape, and possessing nuclei rich in chromatin. All the tumors of this class show in addition to the acinous arrangement solid nests of cells. These cells are no longer cubical, but irregularly shaped, and possess a large vesicular nucleus, which is less rich in chromatin. These cells also invade the acini, which they do not quite fill. The two kinds of cells are readily differentiated by their staining characters.

DESCRIPTION OF THE INDIVIDUAL TUMORS.

1. The first of this series was in an old, white, female mouse (Original iv). The tumor occupied the left inguinal region, measured three and one-half centimeters in diameter and had invaded the lumbar and abdominal muscles. The skin was involved

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[34] Borrel and Haaland, *Compt. rend. Soc. de biol.,* 1905, lviii, 14; Haaland, *Ann. de l'Inst. Pasteur,* 1905, xix, 165.

[35] Erdheim, *Zeit. f. Krebsforsch.,* 1906, iv, 33.

[36] Haaland, *Norsk Mag. for Lægesvidensk.,* 1907, v, 105.

[37] Bashford, Murray and Haaland, *Berliner klin. Woch.,* 1907, xliv, 1194.

[38] Lewin, *Berliner klin. Woch.,* 1907, xliv, 1602.

and ulcerated.   On section there were small cysts and hemorrhagic foci.   The right lung presented a nodule the size of a pin head. The structure was characteristic, being chiefly adenomatous (Plate XVII, Fig. 1), but showing some solid nests of cells and numerous mitotic figures.   As was to be expected, small hemorrhages were also present.   The lung nodule consisted of a metastasis composed mainly of a solid growth of epithelial cells, with, occasionally, the appearance of acini.   It was situated just beneath the pleura and showed numerous mitotic figures.   The stroma was delicate.

From the original tumor 50 mice were inoculated, of which 3, or 6 per cent., developed tumors.   Of these 3, a growth was first noted in two on the nintieth and in one on the one hundred and fiftieth day after implantation.   The transplantation tumors were made up of solid growth of tumor cells (Plate XVII, Fig. 2), between which the stroma was slight and cellular, and sometimes edematous.   There were numerous mitotic figures, many vessels, and frequent hemorrhages.   The necrotic areas were frequent.

In the succeeding generations the tumor preserved its histological characters, but in the sixth generation the necrosis and hemorrhages became more numerous, and acini, similar to those in the original tumor, became common (Plate XVIII, Fig. 3).   The successful transplantations of this tumor never exceeded 40 per cent., and the incubation period averaged ultimately about fifteen days.

2. An old, white, female mouse (Original vi) showed a tumor measuring one and one-half centimeters situated in the left inguinal region.   The skin over the tumor was tense and purple, but free.   The tumor was movable.   On section there were a few hemorrhagic areas in an otherwise grayish-white tissue.   The right lung showed a tumor nodule.

The inguinal tumor was of glandular type, consisting of tubules, some of which were dilated to form cysts, and there were associated solid areas of cells (Plate XVIII, Fig. 4).   The hemorrhagic cysts were apparently of secondary origin, and caused by hemorrhage into necrotic foci.   The lungs showed several small metastases confined about the blood vessels and composed of solid nests of epithelial cells.   In one instance there was observed tubular formation within a small blood vessel.   In one place a growth was observed in an

artery, from which it extended into branches, and in another place several large arteries were occluded by the growth. There were numerous mitotic figures present.

59 mice were inoculated, of which 3, or 5 per cent., developed tumors. The average period of incubation was 120 days. Under the microscope the secondary tumors resembled one another, but differed greatly from the original tumor. The adenomatous structure so striking in the original had been succeeded by solid growths of cells in the secondary tumors (Plate XIX, Fig. 5). Mitotic figures were numerous, and hemorrhages were common in the stroma and between the tumor cells. With material from the first transplantation generation, 143 mice were inoculated, of which 8, or 5.6 per cent., developed tumors. This tumor was carried to the 7th transplantation generation, and the number of successes never exceeded 50 per cent. The microscopical structure of the transplantation generations remained essentially fixed, and consisted chiefly of solid nests of cells with here and there acini and a small amount of stroma. The only striking variation was a somewhat greater development of the cellular and edematous stroma. Hemorrhages appeared not infrequently.

In the second generation one of the tumors presented an appearance somewhat suggestive of a mixture of sarcoma and carcinoma (Plate XIX, Fig. 6). The epithelial cells formed lobules, the central portions being usually necrotic. The lobules were separated from one another by thick bands of very cellular stroma. Large blood vessels were present, the walls of which, in many instances, were composed only of a layer of endothelial cells. It was at first thought that we were dealing here with a transition of the carcinoma into a sarcoma, but the daughter tumors which developed gave no further evidences of this structure.

3. An old, white, female mouse (Original vii) showed a large semi-fluctuating tumor in the right inguinal region. The skin was tense and purple in color, but free. On section the tumor was composed simply of two large cysts filled with a dark brownish fluid. The tissue at the margins was of a grayish color and friable. Under the microscope the preserved tissue showed a structure similar to No. 2.

From this tumor 18 mice were inoculated, of which 3, or 16.6 per cent., developed tumors. The incubation period for two of the three was 45 days, and for the other it was 70 days. Under the microscope these tumors were seen to be composed of closely packed acini, and small hemorrhages were observed. For the second transplantation generation, 61 mice were employed, and 9, or 14.7 per cent., developed tumors. The shortest incubation period was 30 days, and the longest was 150. For the third generation, 56 mice were inoculated and 4, or 7.1 per cent., developed tumors. Of these 4, 3 subsequently were completely absorbed. The histological structure of the transplantation generations was similar to that of the original tumor. The acinous arrangement recurred, but cysts were also present. Mitotic figures were numerous.

4. A very old, white, female mouse (Original xii) presented a tumor measuring one and one-half centimeters in the right inguinal region. The tumor was freely movable. In consistence it was quite firm. The left lung presented at the lower margin a grayish-white wedge-shaped tumor measuring three millimeters in diameter. In the right axilla was a nodule measuring 0.5 centimeter. The original tumor showed under the microscope a somewhat compound structure. While the main portion was composed of solid growths of epithelial cells, there were some acini and small dilated cysts. Where the glandular type occurred, the stroma was fibrous, while in the more solid portions it was delicate. There were some hemorrhages, and mitotic figures were numerous. The lung nodule proved to be a primary cyst-adenoma and will be described elsewhere.

The nodule in the right axilla proved to be composed of the closely packed tubules separated from one another by delicate stroma. The nuclei of the small cells were dense and mitotic figures were not found. No remnants of lymphoid tissue were discovered, and it is probable that this represented an independent tumor formation.

50 mice, 30 white and 20 colored, were inoculated, of which 33 survived two weeks or longer, and one developed tumor, which, however, subsequently underwent absorption. It was detected for the first time 30 days after the inoculation.

5. An old, white, female mouse (Original xiii) showed a tumor measuring one and one-half centimeters in the left inguinal region, which was freely movable. The consistence was firm and the cut surface grayish-white in color. Under the microscope this tumor was almost of pure glandular type (Plate XX, Fig. 7). Microscopical examination of the lungs showed a primary cyst-adenoma which will be described elsewhere.

In the first generation, 80 mice were inoculated and 67 survived. 8 of these, or 11.9 per cent., developed tumors, two of which subsequently underwent absorption. The average incubation period was 70 days. In the second generation, 20 mice were inoculated, and 6, or 33 per cent., developed tumors. The incubation period was 30 days. The structure of the transplantation tumors was similar to the original. In the third generation, 20 mice were inoculated, of which 6, or 33 per cent., developed tumors, and the incubation period was 25 days. In this generation the histological type had altered, so that some of the tumors corresponded to the original, and others were composed of solid lobules with necrotic centers and heavy stroma (Plate XX, Fig. 8). In both, mitotic figures were numerous.

6. A japanese waltzing mouse (Original xv) presented a tumor situated in the front of the neck, almost in the median line, freely movable, and measuring one centimeter in diameter. Under the microscope this tumor was made up of numerous acini and a few cysts. The cells forming the more solid portions had through pressure ·become flattened and spindle-shaped. The stroma was slight, and mitotic figures were numerous. Two small metastases containing acini, but less uniform in structure than the original tumor, were present in the lungs.

40 mice, consisting of 30 white and 10 waltzing mice, were inoculated. None of the white mice developed tumors. Seven of the dancing mice survived longer than two weeks, of which three, or 42.8 per cent., developed tumors. These three died while the tumors were still small, and no further transplantations were attempted. The transplantation tumors resembled histologically the original.

7. A white, female mouse (Original xx), showed, in addition to

two adeno-carcinomata of the type we are describing, other primary tumors, namely, two hemorrhagic cyst-adenomata of the neck and inguinal region, and a primary cyst-adenoma of the lung. One of the adeno-carcinomata, situated on the lower part of the abdominal wall, measured two centimeters, and the other, which was situated in the left inguinal region, measured seven millimeters. These tumors presented a distinctly glandular character, being composed of acini similar to the others of the series. Muscle fibres had in some places been included in the growing tumors.

40 mice were inoculated, of which 28 survived, and 12, or 42.8 per cent., developed tumors. The incubation period ranged from 30 to 60 days. The tumors presented microscopically two types: first, similar to the original; and second, solid cell nests devoid of acini. 40 mice were inoculated in the second generation, and yielded 16, or 40 per cent. of tumors, the average incubation period being 30 days.

8. An adult, light brown, female mouse (Original xxiii) showed a tumor measuring one by one and one-half centimeters, in the right inguinal region, surrounded by subcutaneous fat. It was solid in consistence. In microscopical structure the tumor was of the glandular type. It contained small cysts, and the stroma was fairly well developed. Mitotic figures were numerous.   ·

26 white (?) mice were inoculated, of which 2, or 7.6 per cent., developed tumors, one appearing at the end of 60, the other at the end of 120 days. Except for more numerous hemorrhages, they resembled the original. In the second generation, 20 mice were inoculated, of which 3, or 15 per cent., developed tumors. The structure had now altered: there were fewer acini, a general solid growth of cells, and numerous hemorrhages. Mitotic figures were numerous.

9. A white, female mouse, apparently very old (Original xxiv) showed a tumor measuring 1.3 centimeters on the right side of the thorax, just below the axilla. The structure was solid and lobulated and there was involvement and ulceration of the skin. Under ·the microscope the growth was more solid in type than the other tumors of this series, but a certain amount of acinous formation could be made out. Keratinized foci (Plate XXI, Fig. 9) were num-

erous, the keratinized cells sometimes appearing at the margin of a necrotic area, sometimes surrounded by necrotic material, and sometimes occurring in the preserved parts of the tumor.

50 mice were inoculated with this tumor, but no secondary tumors developed.

10. A yellow, female mouse (Original xxv) presented a tumor measuring one-half centimeter in diameter, situated on the right side of the neck and freely movable. The tumor was friable, and showed minute hemorrhages. There were numerous nodules in the lungs. The microscopical structure was acinous and similar to others in this series, and there were numerous small cysts present containing a serous exudate and sometimes blood. Within some of the cysts there were keratinized areas, showing typical " pearls " (Plate XXI, Fig. 10). Some solid areas of cells also occurred. The lung metastases were numerous and were confined to the blood vessels, which, in many instances, were occluded by them. In other cases the lung tissue was replaced. When the growth occurred in the blood vessels it was usually solid, and mitotic figures were numerous. When the growth in the vessel did not fill it, the outer surface was sometimes covered by endothelium, and in one instance a second independent growth of tumor was observed to have taken place between the old mass, which was covered with endothelium, and the vessel wall. The central portions of some of the large intravascular growths had become necrotic and formed cavities. The extra-vascular growths showed acini and some solid nests and numerous mitotic figures (Plate XXII, Fig. 11).

There was a larger, wedge-shaped nodule in the lung, which proved to be a primary cyst-adenoma, and which will be described elsewhere (Plate XXII, Fig. 12).

Of 50 mice inoculated, 23, or 46 per cent., developed tumors. Of 33 mice inoculated in the second generation, 18, or 54.5 per cent., developed tumors. In structure the secondary tumors resembled the original, except that keratinization was not observed.

HEMORRHAGIC CYST-ADENOMA.

A somewhat special interest is attached to this class of tumors because Ehrlich[39] employed them in an extensive series of experi-

[39] Ehrlich, *Arb. a. d. k. Inst. f. exper. Therapie,* 1906, No. 1, 77.

ments to induce immunity. Having discovered that they were transplanted with greater difficulty than other mouse tumors, and that animals which had successfully resisted their implantation presented an increased resistance to the inoculation of other tumors readily transplantable to normal mice, he employed them for producing this refractory or immune state. On the other hand, some investigators have succeeded in transplanting this type of hemorrhagic tumor. Hertwig and Poll[40] report a successful instance, and Tyzzer[41] reports a successful instance, so far as the first generation is concerned, while Gierke[42] reported that of 47 such tumors, 38 were transplanted successfully by him. He gives the details of his experiments with 35 separate tumors. Of these 35, 29 were transplantable, and of the 29, 15 became extinct as follows: 8 in the first generation, 5 in the second, and 2 in the third. In only one instance was he successful in carrying a tumor of this class into the fourth transplantation generation. Gierke states that of 2,851 mice which survived for a sufficient time, 187, or 6.5 per cent., developed tumors. The highest original result ever attained was 33 per cent., and the highest subsequent one was 70 per cent. of the tumor fragments implanted.

The tumors of this class can frequently be detected from their gross appearances, since the skin covering them is usually tense and purplish in color, and the section of the tumor shows large cysts containing bloody contents and little original tumor tissue. They are as a rule incapsulated. Under the microscope the more solid portions are composed of regular acini, but everywhere there are cysts of variable size, produced through dilatation of the acini and through necrosis of the tumor. The cells which line the acini are usually cubical and rich in chromatin, while those lining the cysts are usually flattened. The stroma is usually delicate in character, but may be thicker and denser, or edematous. Large hemorrhagic foci are scattered through the sections, as well as large thin-walled vessels. The walls of the vessels are so thin at times that they

[40] Hertwig and Poll, *Abhand. d. k. preuss. Akad. d. Wissensch,* Berlin, 1907, i, 1.

[41] Tyzzer, E., *Fourth Report of the Caroline Brewer Croft Fund Cancer Commission,* 1907, 27.

[42] Gierke, E., *Scientific Report of the Imperial Cancer Research Fund,* 1908, No. 3, 115.

are made out with great difficulty, and thus it becomes uncertain sometimes whether the blood is contained within the vessels, or within a cyst. The mitotic figures are usually infrequent.

In our series there were ten tumors belonging to this class, from which 365 mice were inoculated. However, as 61 died within the first two weeks, only 304 survived long enough to permit the development of tumors. Of these, 21, or 6.9 per cent., developed tumors and later seven of the tumors became absorbed. Of these ten tumors, seven, or 70 per cent., gave transplantation tumors in the first generation. Of these seven, two failed to develop tumors in the second generation, and one in the third, while the remaining four were transplanted successfully through successive generations. In one of the ten mice a lung metastasis was present, although in three others primary lung tumors occurred.

1. The tumor measured one and one-half centimeters in diameter, and appeared in the right inguinal region of a female mouse (Original i) kindly presented to us by Dr. F. C. Wood of New York. The skin covering the tumor was tense, partially free of hair, and purplish in color. The tumor was fluctuating on palpation, and on incision, a large amount of dark fluid escaped from a smooth-walled cyst on the inner wall of which papilliferous masses were attached.

The solid portion of the tumor was suspended in normal salt solution, after being cut into minute fragments, and 15 mice were inoculated under the skin. None developed tumors. Under the microscope the tumor was found to be composed mainly of cysts in the tissue, between which there were regular acini. Hemorrhages of different durations had taken place into the cysts. A small number of mitotic figures were seen, and occasionally solid masses of cells.

2. A brown, female mouse, 15 months old (Original ii), showed a tumor on the left side of the body, just below the axilla, which measured 1.75 centimeters. The tumor was grayish-white in color, lobulated and presented on section many small hemorrhagic cystic areas. On the opposite side of the body in the same general position a nodule 4 by 6 millimeters occurred. It was loosely attached

to the skin, and is described under the cyst-adenomata.   Under the microscope the larger tumor was composed of closely packed tubular acini, lined by a single layer of epithelial cells.   Sometimes the epithelium was arranged in several layers, or entirely filled the spaces.   Hemorrhages were scattered through the tissue, being contained within cyst-like spaces.   The blood vessels presented very thin walls and appeared often as sinusoids.   One of the mammary glands adjacent to the nodule showed much dilated tubules, filled with a glandular eosin-staining detritus.

41 mice, of which 19 were brown and 22 white, were inoculated with fragments which had not come into contact with saline solution, and none of the 19 brown mice developed tumors, while 21 of the 22 white mice developed them.   One of these was first noticed on the 150th and the other on the 180th day after inoculation.   From the former, 20 white mice were inoculated, one of which died in a few days, and of the remaining 19, 3, or 15.7 per cent., developed tumors, one of which subsequently underwent absorption.   The incubation period in this series ranged from 60 to 90 days.

Microscopical examination showed the transplantation tumors to be more solid in character than the original, to contain hemorrhages and to show quite numerous mitotic figures.   Some acini were present, and the epithelial cells in the two kinds of structures resembled the original.   Microscopical examination of the tumors of the second transplantation generation showed marked changes as compared with the original.   There were still present numerous acini and cysts, but the latter were produced by a disintegration of necrotic cells.   The tumors were also more lobulated, and there were ingrowths from the sides of the lobules of a somewhat variable character.   However, the larger part of the tumor consisted of a solid growth of cells.   The stroma was cellular and well developed in places, but the blood vessels were still large and thin-walled.   Hemorrhages were not infrequent, but not so common as in the original tumor.   Mitotic figures were not infrequent.

In the third generation, forty mice were inoculated, yielding one slowly growing tumor.   In the fourth generation, twenty were inoculated yielding seven tumors, or 35 per cent.   Subsequently, six

of these seven were absorbed. Further transplantations were not attempted.

3. White, female, apparently old mouse (Original iii) showed a tumor on the left side, just below the axilla and extending in front almost to the median line. The skin was tense and purplish in color. On removal the tumor was dark brown, and on section exuded a dark fluid. It measured three by one and one-half centimeters. Under the microscope it proved to be composed of lobules consisting of closely packed acini. The centres of the lobules usually were cystic, and the cysts contained an eosin-staining, homogeneous substance and blood. Some solid nests of cells existed, and mitotic figures were numerous. Muscle fibres were contained in the upper pole of the tumor.

59 mice were inoculated, of which 4, or 6.7 per cent., developed tumors. The first growth was noticed on the 120th day, and the later growths on the 150th and 180th days. All but small parts of three of the tumors were excised, and used to inoculate 34 mice. The fragments of tumor left behind underwent subsequent absorption. Of the 34 mice of this series, one developed a tumor at the end of 60 days. As the tumor was growing very slowly, an incision was made in it with the idea that it might be stimulated to more active growth, but after the operation there was complete absorption. The microscopical examination of the first transplanted tumors showed cystic growths in which the cysts were filled with blood (Plate XXIII, Fig. 13). The general structure was acinous. The microscopical examination of a fragment removed from the second generation transplantation showed a papillomatous growth with cysts and some hemorrhages (Plate XXIII, Fig. 14).

4. White, female, evidently old mouse (Original viii). The tumor had been partially eaten away by another mouse in the same cage. It was on the left side and partially surrounded the vagina. In color it was dark brown, and contained several cysts in which there was a dark brown fluid. Microscopical examination showed the tumor to contain a large amount of dense stroma, and to be divided into small lobules, which themselves were made up of acini. Hemorrhages were common in and between the latter.

30 mice were inoculated with fragments of this tumor, but no secondary tumors developed.

5. White, female mouse (Original ix) showed a tumor measuring two centimeters on the right side just below the axilla. The skin was tense and purplish in color. The external portion of the tumor was composed of a large cyst and there were smaller cysts in other portions. Under the microscope the tumor was found to be composed almost entirely of cysts which in places were being replaced by granulation tissue. The sections removed from the deeper portions alone showed traces of the original tumor. Many hemorrhages were present, and the blood vessels were numerous.

40 white mice were inoculated from this tumor, of which 25 died in less than two weeks. Of the 15 surviving, one, or 6.6 per cent., developed a tumor, and the incubation period was 60 days. This mouse died during the hot summer months, and decomposition set in so rapidly that no further attempts were made to transplant it to other mice. Microscopical examination showed the transplanted tumor to be like the original.

6. White, female and apparently old mouse (Original x) showed a tumor measuring two centimeters in the left inguinal region. The skin over it was tense and purplish in color. On section, the tumor was composed almost entirely of cysts containing a dark fluid (Plate XXIV, Fig. 15). The cyst walls were thin, and there was little of the solid tumor tissue left except at the margins. A small nodule was present just beneath the pleura in the left lung, which proved to be a primary cyst-adenoma. In microscopical appearances this tumor was similar to others of this class, and cysts and hemorrhages were everywhere present. There were numerous mitotic figures.

30 mice were inoculated, of which 9 died within a few days. Of the 21 surviving, 2, or 9.5 per cent., developed tumors. The incubation period was 60 days. One of the two was ultimately absorbed, and the remaining mouse with tumor died before it became large enough to be used for transplantation.

7. A brown, female and apparently old mouse (Original xi) showed a tumor at the right axillary region, measuring two by one and one-half centimeters. It was composed of a large cyst externally, and was filled with a dark, thick brownish fluid. The deeper portion was grayish-white in color and firm in consistence. There

were numerous lung metastases. Excepting for the thickness and density of the stroma, this tumor was very similar to others previously described. In some places there was a tendency towards development of papillary outgrowths in the walls of the lobules. The lobules for the most part were filled with closely packed acini, and the stroma contained many large phagocytic cells filled with blood pigment. Mitotic figures were not numerous. The lungs were thickly studded with metastases, confined chiefly to the blood vessels. Few of the larger vessels had escaped invasion. The growths in the lung resembled the original, even to the formation of cysts and the presence of hemorrhages. This animal died during one night and when it was examined the next morning there was already beginning decomposition. 40 mice, of which 10 were white and 30 light brown, were inoculated, and no tumors developed.

8. An adult, white, female mouse (Original xvi) presented a tumor in the right inguinal region, measuring two and one-half centimeters. The skin was attached and ulcerated. The tumor was gray and contained numerous hemorrhages. A single small cyst was observed. The left lung contained a small primary tumor. Under the microscope the superficial tumor was distinctly hemorrhagic in type, but less homogeneous in structure than the others of this class. The arrangement of the epithelium was highly varied. Sometimes it was glandular and formed large cysts; at other times there were lobules filled with a solid growth. Necrosis was common, and hemorrhages frequent, and the blood vessels were large and possessed thin walls. In some places the acini had become greatly dilated, and into their lumina larger cells with vesicular nuclei had penetrated.

From this tumor 40 white mice were inoculated, of which 12 died in a few days. Of the 28 surviving, 6, or 21 per cent., developed tumors; the incubation period was 20 days. Three of these tumors subsequently underwent absorption. The microscopical structure of the secondary tumors agreed with the original.

Of 37 mice inoculated from the first transplantation generation, 13, or 35 per cent., developed tumors, of which 8 subsequently underwent absorption. The incubation period was about 25 days.

The glandular or acinous structure present in the original tumor could be made out hardly at all in the tumors of this generation. The stroma had become well developed, and subdivided the tumor into small lobules, which were filled with cells showing no definite arrangement. A striking feature was the strong development of the stroma. Necrosis of the central portion of the lobule was common.

20 mice were inoculated for the third generation, of which 5, or 25 per cent., developed tumors. Three of these subsequently underwent absorption. The incubation period was about 25 days. The structure remained about the same as the previous generation. 28 mice were inoculated in the fourth generation, of which 11, or 39.2 per cent., developed tumors. Eight of these subsequently were absorbed. Microscopical appearances of this generation were similar to the original tumor.

9. Dark, gray, female mouse (Original xix), among the stock animals of the institute, showed a tumor 1.3 centimeters in diameter, situated beneath the skin of the lower portion of the abdomen, to the left of the middle line. It had slightly invaded the muscles of the thigh. The surface was irregular. The tissue was hemorrhagic and there were numerous cysts. In appearance it was found to be composed of numerous cysts and necrotic areas containing hemorrhages, and areas of epithelial cells showing little definite arrangement. Mitotic figures were common. The blood vessels were greatly dilated.

From this tumor 20 mice, 10 of the color of the original, and 10 white, were inoculated. Of the 10 white mice inoculated, 2 died in a few days, and of the 8 surviving, one developed a tumor after 45 days, which later became absorbed. One of the ten colored mice developed a tumor after 120 days which was used for further inoculation. Under the microscope this tumor was less adenomatous in structure than the original. It showed hemorrhages and necrotic areas. From this tumor 15 mice (colored?) were inoculated, of which 2, or 13.3 per cent., developed tumors. In one the incubation period was 160 days and in the other, 90. The structure remained about the same as in the preceding generation. Further transplantation was not undertaken with this tumor.

10. White, apparently old, female mouse (Original xx) showed on neck anterior to the right of the median line, a tumor two and one-half centimeters in diameter. The surface was gray and mottled with dark brown, the latter portions being cystic. Under the microscope this tumor was adenomatous in structure, consisting of acini intermingled with solid masses of epithelial cells. Areas of necrosis and hemorrhage occurred, the former being more frequent. The acini were frequently dilated into cysts, some of which contained a homogeneous eosin-staining material, and others, blood. There were numerous mitotic bodies. The blood vessels were widely dilated.

50 mice were inoculated, of which 5, or 10 per cent., developed tumors. One of these later underwent absorption. The incubation period was 30 days. The structure of these tumors differed from the original in that the stroma was far better developed and was very cellular (Plate XXIV, Fig. 16). Large hemorrhagic areas occurred and the acinous arrangement had almost completely disappeared. There were large lobules composed of central cavities containing cells, and hemorrhages were common.

40 mice were inoculated from this generation, of which 3, or 1.5 per cent., developed tumors, all of which underwent absorption before they reached a size large enough to be used for further transplantation.

<div align="center">CYST-ADENOMA.</div>

Of this class there were two tumors, and since they occurred in mice in which the other classes of tumors were present, and which were of larger size, they were not submitted to transplantation.

1. The first of these, which occurred in Original ii, was situated on the right side of the thorax and measured .6 centimeter in its greatest diameter. In form it was flat and freely movable. The tumor with which it was associated was a hemorrhagic cystadenoma.

2. The second of these tumors was in Original v. It was situated on the left side of the thorax, was flat, and measured 0.5 centimeter in its greatest diameter. The tumor with which this was associated belonged to the class termed by Apolant solid carcinoma.

In histological structure these growths were very similar to one another. They were made up of cysts developed from dilated acini, and lined by a single layer of cells, some of which were flattened. The cysts, however, frequently contained a homogeneous, eosin-staining material. No mitotic figures were discovered. The stroma was delicate in character.

The possibility, of course, exists that these second tumors of smaller size were metastases. The type, however, of the smaller tumors was wholly different from the larger ones, and approached more nearly the typical adenomatous form than did the others. Since each was distinctly incapsulated, and there were no mitotic figures discovered, and no evidences available of, growths having developed in the lymphatic glands, they were regarded as independent tumors.

ALVEOLAR CARCINOMA.

According to Apolant, in this type of tumor, no acini, strictly speaking, are present, but the tumor consists of lobules containing alveoli closely packed with epithelial cells. One such tumor came under observation among our series. It occurred in a white female mouse (Original v), was situated in the left inguinal region and measured 2 by 2 centimeters. Neither the skin nor the abdominal muscles were involved in it. Its central portion was necrotic, consisting of a cheesy mass, while the cut surface presented a granular appearance. Its external contours were irregularly lobulated, and nutrient blood vessels radiated over its surface. Near the left axilla there was a flat nodular growth measuring 0.5 centimeter, and presenting the same appearances as the large tumor.

Microscopically this tumor consisted of lobules filled with closely packed epithelial cells, many of which through pressure had become flattened and spindle-shaped. These solid masses of cells bore a certain resemblance to spindle-cell sarcoma. Where the lobules were large, the central portions were necrotic. Mitotic figures were numerous. It should be stated that at one point where the stroma was unusually well developed a few acini occurred, and that the second flat nodules presented the characters of cyst-adenoma.

49 mice were inoculated from the alveolar tumor, of which 10,

or 20 per cent., developed tumors. The average incubation period was 70 days. This tumor is now in the sixth generation, and the highest percentage of implantations thus far obtained has been 59 per cent. The structure of the transplanted tumors is similar to that of the original.

MOLLUSCOIDAL TUMOR.

A tumor differing greatly from all others which have come to our attention will now be described. It resembles closely a tumor described by Haaland,[48] and, owing to a certain resemblance to the lesions of *molluscum contagiosum,* he termed it "tumeur molluscoide." He describes the tumor as being composed of lobules with rounded bases at the periphery which radiate from a common centre in the manner of the spokes of a wheel. The lobules are broader at the periphery, and the central portions of them show frequently the presence of keratin. The tumor which came to our attention agrees closely with this one, although the degree of keratinization was somewhat less. According to Haaland's view, the tumor developed from hair follicles, and we are of the opinion that our tumor took its origin in the same structures. He inoculated twelve mice, but in no case did he get a secondary growth.

This tumor occurred in a full grown, brown, female mouse (Original xxii). The growth was situated in the right inguinal region, and measured 2 by 3 centimeters. It had become attached to the skin, and ulceration was beginning. The tumor also penetrated the abdominal muscles and projected slightly into the abdominal cavity. It was grayish-white in color, rather firm, and showed one hemorrhagic area. There were no cysts. In the upper portion of the lower lobe of the left lung a nodule measuring one millimeter in diameter was present.

At the margin of the tumor, where it invaded the neighboring tissues, the cells were arranged in columns, consisting of solid growths of epithelial cells (Plate XXV, Fig. 17). Where these columns were cut transversely, they presented somewhat the appearance of tubules. The general tendency of the tumor was to grow in such columns. Tubular formations were also present, some of which

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[48] Haaland, *Ann. de l'Inst. Pasteur,* 1905, xix, 163.

branched and appeared to end blindly in solid masses of cells. These tubules presented several layers of cells. Mitotic figures were very common. Throughout the section irregular masses of kerato-hyalin occurred (Plate XXV, Fig. 18). They usually occupied the central portions of the solid lobules which were found both in the periphery and central portions of the tumor. As a rule, these areas were more abundant in the solid strands themselves, but they also occurred in the tubular formations, the lumen of some of which were filled with it. The nodule in the lung proved to be a primary cyst-adenoma.

50 mice were inoculated with the large tumor, but none developed secondary tumors.

PAPILLARY CYST-ADENOMA OF THE LUNG.

The class of tumors to which the primary lung tumors to be recorded belong was first described by Livingood,[44] who found it accidentally in the lung of a mouse dying of an experimental bacterial infection. Much later Haaland[45] reported five instances of this tumor, but the most complete description in the literature is that of Tyzzer,[46] who described a series of twelve such tumors.[47] It is noteworthy that in Tyzzer's series of cases, six of the animals presented tumors in other parts of the body, or in other words, were the subject of multiple primary tumors. Moreover, the circumstance should be mentioned that in the examples described by Haaland the mice had previously been inoculated with tumor fragments, so that he has considered the possibility of their origin from the inoculated material. Murray[48] has recently reported finding this type of lung tumor, but he does not state with what frequency.

Our observations are limited to nine primary tumors of the lung

[44] Livingood, L. E., *Johns Hopkins Hosp. Bull.*, 1896, vii, 177.

[45] Haaland, *Ann. de l'Inst. Pasteur*, 1905, xix, 165.

[46] Tyzzer, E., *Fourth Report of the Caroline Brewer Croft Fund Cancer Commission*, 1907, 27.

[47] In a later report (*op. cit.*) Tyzzer states that he has met with 37 mice showing primary lung tumors. In one family of 25 mice, four examples of primary adeno-carcinoma of the lung were found; and in another family of 100 mice, springing from a mouse with an adenoma of the lung, 15 mice developed tumors.

[48] Murray, *Scientific Report of the Imperial Cancer Research Fund*, 1908, No. 3, 69.

of this type, one animal of this series presenting two such tumors. These tumors were distributed as follows: two of them occurred in mice in which there were hemorrhagic cyst-adenomata, four in mice with adeno-carcinomata, one in a mouse with multiple superficial tumors, and one in a mouse in which the superficial tumor was a spindle-cell sarcoma. It was this last animal which showed two primary lung tumors, one in each lung. With one exception, the tumors were all too small to be used for purposes of transplantation.

1. The first of these tumors which we came across was in Original Mouse xii. It was located in the lower margin of the left lung, was grayish-white in color, and measured three millimeters in diameter. It projected slightly above the surface of the pleura, and was sharply outlined. Its microscopical appearance consisted of irregularly projecting folds of connective tissue containing cavities which were lined by columnar epithelial cells, the outlines of which were sharp (Plate XXVI, Fig. 19). A small bronchus, the lumen of which was almost occluded by the growth, approached the periphery of the tumor. The epithelial cells of the tumor and the bronchus were almost indistinguishable. The central part had begun to degenerate and contained a greater amount of fibrous tissue. The cavities of the tumor contained large cuboidal cells, undergoing degeneration. Mitotic figures were not discovered. The pulmonary tissue immediately adjacent to the tumor was collapsed. The epithelial cells covering the papillary outgrowths of connective tissue formed a single layer, and the stroma was small in amount.

Serial sections showed the growth to be wedge-shaped, and the apex to consist of two or three papillary outgrowths from the wall of a bronchiole. From the front of the organ the growth extended along a bronchiole into the larger bronchi and the surrounding tissue.

2. The second tumor of this type occurred in Original Mouse x. The nodule measured 0.5 millimeter and was located in the upper lobe of the left lung, projecting slightly above the surface of the organ, and being sharply outlined. Under the microscope this tumor proved to be more solid than No. 1. It also invaded the pulmonary alveoli to a larger extent, which it appropriated to supply its framework. The individual cells were round and the nuclei were rich in chromatin. Mitotic figures were not discovered. In the spaces

between the papillary outgrowths numerous rather large cuboidal cells were present.

3. The third tumor was found in Original Mouse xiii. It was not seen at the original examination of the animal, and was detected subsequently in the study of sections of the lungs. The tumor was located in the central portion of the upper lobe of the right lung. In structure it resembled lung tumor No. 1, except for the epithelial cells, which were cubical in shape (Plate XXVI, Fig. 20). The general structure of the tumor was also more compact, since the spaces between the papillary projections were smaller. Serial sections showed the tumor to be conical in shape, and the apex of the cone to lie in close relation with and apparently to spring from a terminal bronchiole (Plate XXVII, Fig. 21).

4. This tumor was found in Original Mouse xvi. On the anterior surface of the lower lobe of the left lung, a grayish-white mass one millimeter in diameter existed. It projected above the pleura and was sharply outlined. Under the microscope this mass was found to be composed of papillary outgrowths covered by a single layer of epithelial cells of cubical form. The cells were smaller than in the preceding tumors of this type. A special feature of this tumor was the presence of a homogeneous material separating the papillary projections in the more solid portions, and filling the small cystic spaces. Mitotic figures were not discovered. There was compression of the surrounding lung tissue.

5. The next tumor of this class was found in Original Mouse xx. It was situated in the upper lobe of the right lung and measured one · and one-half centimeters in diameter. Its structure was very similar to those already described. The central portion was fibrous, because of degeneration of that part of the tumor. This fibrous tissue contained clefts in which there had been fatty acid crystals, and the clefts were surrounded by foreign body giant cells. The surrounding lung tissue was collapsed.

6. The next tumor of this type occurred in Original Mouse xxii. It measured one millimeter in size and occurred in the upper portion of the lower lobe of the left lung. It resembled in structure the previous tumors described.

7. The seventh of the series occurred in Original Mouse xxv. In

this animal the lung showed several nodules, two of which were situated near the apex of the right lung, and measured about two millimeters in diameter. One of these only was found to be a primary pulmonary tumor, the others being metastases of the superficial adeno-carcinoma. The primary tumor corresponded to the general tumors of this type and differed greatly from the metastatic nodules.

8. In Original Mouse xxvi two primary lung tumors were present. One was found in the upper lobe of the right lung and the other in the upper lobe of the left lung. They measured about one millimeter in diameter. The superficial tumor with which they were associated was a spindle-cell sarcoma. In structure they were quite similar to others of this class (Fig. 12).

SARCOMA.

The literature does not contain, as far as I have been able to see, records of the successful transplantation of primary sarcoma of the mouse into other mice, although sarcomata of rats have been in several instances transplanted successfully. Ehrlich and Apolant[49] have reported the finding of two mixed tumors composed of sarcomatous and carcinomatous elements in the first generation, and Murray[50] alludes to an instance of a spindle-cell sarcoma found by Jensen in a mouse sent to him by Bashford.[51] In the latter instance the growth surrounded one of the kidneys and although it was transplanted into a large number of mice, it gave rise to no implantation tumors. In the instances mentioned as having been reported by Ehrlich and Apolant, they regard the tumors as having been original carcinomata, which were being transformed during the original generation into sarcomata. Ehrlich and Apolant,[52] Loeb,[53] Liepman[54]

[49] Ehrlich and Apolant, *Berliner klin. Woch.*, 1907, xliv, 1397.

[50] Murray, *Scientific Report of the Imperial Cancer Research Fund*, 1908, No. 3, 73.

[51] Bashford and Murray, in a later article (*Proc. of the Royal Med. and Chir. Soc. of London*, Series B, 1909, lxxi, 311), mention a melanoma or melandric sarcoma of the ear of a mouse, but do not allude to transplantation.

[52] Ehrlich and Apolant, *Berliner klin. Woch.*, 1905, xlii, 871.

[53] Loeb, *Med. Bull.*, 1906, xix, 113.

[54] Liepman, *Münchener med. Woch.*, 1907, liv, 1345.

and Bashford[55] all report examples of the transformation of carcinoma of the mouse into spindle-cell sarcoma. In all of these instances excepting one, namely, that of Loeb, the changes occurred in relatively late generations, whereas the example reported by Loeb is the first instance in which the change began in the first generation. I have observed two instances of what appeared to be primary sarcomatous superficial tumors of the mouse. In both instances tissue was examined from all parts of the tumors in order to exclude as far as might be possible a mixed carcinoma and sarcoma in the first generation. No evidence of epithelium or of cancerous tissue was detected.

1. The first of these tumors was found in a white, adult, female mouse (Original xviii). It was located on the left side immediately inferior to the foreleg, and extended over the lower ribs. It measured three centimeters in diameter. The skin was invaded, and there was an ulcer measuring half a centimeter in diameter which was superficial and covered with a scab. The tumor was grayish-white in color, firm in consistence and had extended into the muscles about the ribs and projected into the pleural cavity as a flat mass with a concave surface. On section it presented central necrotic masses. There were no metastases, but both ovaries were enlarged, firm, oblong in shape and about eight times their normal size.

Under the microscope this tumor proved to be a spindle cell sarcoma, possessing highly invasive properties and insinuating itself between the voluntary muscle fibers (Plate XXVII, Fig. 22). The ribs were surrounded by tumor cells, but were not invaded themselves. The enlarged ovaries were the seats of papillary cyst-adenomata, which will be described in another place.

60 mice were inoculated from the superficial tumor, of which 45 survived two weeks or longer. In one instance a tumor developed, which was detected on about the 70th day. The tumor grew slowly and a portion of it was removed for examination with a view of stimulating the growth of the remainder. The mouse succumbed soon after the operation, before the growth had reached a size large enough for further transplantation.

2. This tumor occurred in Original Mouse xxvi. It was situated

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[*] Bashford, *Berliner klin. Woch.*, 1907, xliv, 1238.

on the left side of the chest just below the fore leg, measured three centimeters in diameter, was flattened and had invaded the skin. A small ulcer had appeared which was also attached to the chest wall, although it had not penetrated to the pleura. It was grayish-white in color and rather firm in consistence. Tissues immediately surrounding the ulcer were hemorrhagic, while around the main tumor they were edematous. A small nodule measuring one millimeter in diameter was found in the lower portion of the upper lobe of the right lung. The corresponding portion of the left lung showed a similar nodule. These were primary tumors which have been described elsewhere. Both ovaries were enlarged to about five times the normal size.

The microscope showed this tumor to be also a spindle cell sarcoma which had invaded the adjacent muscle fibers. Many of these, or remains of them, were contained in the mass (Plate XXVIII, Fig. 23). A large number of eosinophilic cells were scattered through the tumor. The left ovary was the seat of a cyst adenoma to be described elsewhere.

40 white mice were inoculated from the superficial tumor, of which 14, or 35 per cent., developed tumors. The average incubation period was 30 days. The tumors presented the same histological character as the original, and are now growing in the second generation.

LYMPHO-SARCOMA AND HODGKIN'S DISEASE.

The first tumors of this class were described by Haaland.[56] He described in all six examples of mice in which the lymphoid tissue throughout the body was so greatly increased that he concluded that the condition consisted not of tumor formation in the true sense so much as an hypertrophy or hyperplasia of the preëxisting lymphoid structures. He inoculated other mice with the lymphatic material without results. On the other hand, through having kept mice in a cage with an animal affected in this way, he observed five similar formations in the mice introduced into the cage within a period of two years. Tyzzer[57] reports two instances of a condi-

[56] Haaland, *Ann. de l'Inst. Pasteur*, 1905, xix, 197.
[57] Tyzzer, *Fourth Report of the Caroline Brewer Croft Fund Cancer Commission*, 1907, 27.

tion somewhat resembling the condition described by Haaland, but he states that they presented the appearance of a "well-defined primary growth without appreciable hyperplasia of the lymphoid tissues elsewhere in the body."[58]   Murray[59] reports four similar instances.   In two, a general hyperplasia of the lymphoid tissue existed, and in two, the tumors were more localized.   Both Tyzzer and Murray failed to transplant successfully the tumors to other mice.

1. The first example which I observed occurred in a white, female mouse (Original xvii).   On each side of the neck were masses measuring 1.2 centimeters in diameter which were freely movable and not attached either to the skin or to the deeper tissues. The axillæ contained masses measuring from five to seven millimeters and the right inguinal region, a mass measuring five millimeters.   All of these presented the same naked eye appearances. They were grayish-white in color, quite uniform and not lobulated. The liver was mottled, owing to the presence on the usual dark ground of many minute grayish points.   The upper pole of the right kidney contained a grayish nodule measuring one and one-half millimeters and the left kidney showed two smaller nodules.   Two small masses occurred in the retro-peritoneal tissues just behind the left kidney.   Two masses measuring seven millimeters in diameter were attached to the mesentery.   The lungs also contained several small foci, gray in color and sharply outlined.   The spleen was considerably enlarged and mottled.

Under the microscope the structure of all of the different masses described proved to be the same.   The masses were made up almost exclusively of small round lymphoid cells possessing a comparatively large pale vesicular nucleus and a narrow rim of cytoplasm. The cells were of the type found in the germinal centres of the lymph follicles.   The stroma was delicate except where the larger blood vessels coursed.   Mitotic figures were not numerous.   In addition to the larger cells described, others of the usual lympho-

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[58] Tyzzer states in his later paper (*op. cit.*) that he has met with 10 mice showing lympho-sarcoma; and Bashford and Murray in their last report (*op. cit.*) record another instance of generalized lympho-sarcoma.

[59] Murray, *Scientific Report of the Imperial Cancer Research Fund,* 1908, No. 3, 74.

cytic type were present. Small necrotic areas occurred here and there in some of the nodules.

The lungs showed much more replacement of the pulmonary tissue by the lymphoid growth than was noticeable to the naked eye. When the lymphatic nodules were small they were confined to the perivascular and peribronchial tissues, but the larger masses also invaded the pulmonary tissue. In addition, small accumulations of cells of the same general type occurred beneath the visceral pleura. Mitotic figures were fairly common. In other respects the lungs were congested and edematous, and contained numerous phagocytic cells containing pigment. It may be remarked that the pulmonary condition was similar to that described by Haaland.

The spleen was greatly modified, since both the pulp and the follicles were diffusely infiltrated with the lymphoid cells in such a way as to make the normal structure of the organ indistinct. Many myeloid giant cells were present, as is not uncommon in the spleen of the normal mouse. The sinusoids of the spleen were greatly distended with the cells, which, on the whole, appeared larger than the average. Mitotic figures were numerous.

The liver also exhibited an extensive invasion with the new cells. In some places the growth of these cells had replaced the normal tissue to such an extent that nothing of the liver structure remained visible. In other places the cells had grown into and distended the sinusoids, producing a compression of the rows of liver cells. Sometimes the new cells had spread out diffusely and at other times they had been confined to definite loculi. The interlobular tissue was more affected than other parts.

The new cells in the kidney were confined almost entirely to the cortex of the organ. The growth in the cortex was intertubular and periglomerular; in the medulla it surrounded the vessels only.

100 mice were inoculated with the material from the superficial masses, 70 were inoculated from the masses in the neck, 10 from the mass in the left axilla, 10 from the mass beneath the right pectoral muscles, and 10 from the mass in the right inguinal region. At the end of two weeks 60 of these mice remained alive, of which one developed a tumor. This growth was first noticed 120 days after the inoculation. When the growth had attained the size of

four by seven millimeters, a portion was removed for further inoculation and for histological study. The fragment which was left behind underwent absorption. 20 mice were inoculated with the excised fragment, but none developed tumors. Under the microscope the structure of this implantation nodule was in all respects similar to the original growths, except that the mitotic figures were more numerous, as were the capillaries. The stroma presented the character of the stroma of the original nodules.

2. The second example appeared in a white, female mouse (Original xxi) which showed a superficial tumor in the right inguinal region. The mass measured one centimeter and was freely movable. A portion of the tumor was removed by operation. It was grayish-white in color and somewhat lobulated. The fragment of tumor left behind did not increase appreciably in the next 14 days, the period during which the mouse survived. The autopsy showed a small mass one millimeter in diameter in the upper margin of the lower lobe of the right lung. It appeared to be a metastasis. Unfortunately the tissues removed at the autopsy were lost, and only the portions removed by operation remained for further study.

This tumor was composed for the most part of cells of the lymphoid type which were somewhat smaller in size than in the preceding case. While many of the cells resembled the lymphocyte, others were somewhat larger and possessed pale vesicular nuclei. The stroma consisted of a delicate reticulum enclosing small masses of cells. In the periphery there were many capillaries. Mitotic figures were not very numerous. Two appearances are highly important: in the first place there were many eosinophilic cells scattered through the tissue, and next there also occurred here and there, and particularly in the more central parts of the growth, large areas composed of a reticulum coarser than the usual reticulum and containing spaces enclosing large endothelioid cells, of which some were multinuclear. In addition these areas contained large and small lymphoid cells, a smaller number of eosinophilic and a large number of plasma cells. The endothelioid cells presented irregular margins and contained a large amount of cytoplasm that was strongly eosinophilic. The nuclei of these cells were either vesicular or solid and dense. A certain number of mitotic figures were present

among these cells. The large multinucleated or giant cells showed nuclei which were usually vesicular and contained a coarse chromatin network which was disposed in an irregular fashion in the cytoplasm. In these areas the lymphocytes were few in number, and the other cells relatively numerous. A small number of cells containing basophilic granules were also present.

The description just given indicates that the tumor in this animal possessed the histological characters of Hodgkin's disease, as has been described by Reed, Longcope and others, in human beings. It is true that the eosinophilic cells were perhaps less numerous in the mouse nodule than is the case usually in the human disease, but Longcope has pointed out that in the later stages of the disease in human beings the eosinophilic cells also diminish. Whether, therefore, we are to regard the nodule in the mouse as representing a late stage of the disease, or whether in this animal the eosinophilic cells tend to be less numerous, must be left undecided.

OVARIAN TUMOR.

I have observed two examples of tumors affecting the ovaries, and it is interesting to note that in both cases the large superficial tumors also present consisted of spindle-cell sarcomata.

1. The first example occurred in Original xviii. In this white mouse both ovaries were enlarged, firm in consistence, oblong in shape, and about eight times the normal size. Under the microscope the two organs presented the same appearances which were interpreted as representing papillary cyst-adenomata. There had taken place a great increase of the epithelial cells, which formed solid masses somewhat compressed and elongated into spindle cells and cysts of different sizes, usually small and more or less occupied by the papillary outgrowths from their walls. These outgrowths developed from narrow or somewhat thicker pedicles and spread out into a fan-like structure. The epithelial cells which covered the papillæ and the walls of the cysts were rich in cytoplasm and the nuclei of the cells were usually vesicular but sometimes dense. The more solid portions of the organs were at one time cystic but the cysts became occluded by the ingrowth of the papillæ. Acini

possessing a distinctly granular form and arrangement also occurred. Mitotic figures were rarely seen, but appearances suggestive of direct cell division were more frequent. Hemorrhage had taken place into some of the cysts. Sections stained by Mallory's connective tissue method revealed a delicate basement membrane of connective tissue surrounding the small cysts and the more solid portions as subdivided into small areas of quite definite form. These latter areas were filled with epithelial cells of a granular quality resembling somewhat the lutein cells of the ovary.

2. The second example of ovarian tumor occurred in Original Mouse xxvi. The left ovary was about five times the normal size and showed under the microscope numerous large cysts separated by smaller ones and the tubules of the ovary. The cysts and tubules were lined by high columnar epithelium, but cilia were not demonstrated. In some of the larger cysts the lining epithelium had become flattened. The tissue separating the cysts was composed to a large extent of smooth muscle cells arranged in strands or in bundles. Sections stained by Mallory's phosphotungstic acid and hematoxylin brought out the myoglia fibrils. The nuclei of these muscle cells were large and vesicular and showed a few mitotic figures. In certain portions the granular structures·were few and the muscular fibres many, so that the appearance presented was that of a leiomyoma.

CONCLUSIONS.

There have come to our hands within a period of about two years and as a result of relatively non-strenuous efforts 26 mice which had developed spontaneous tumors, and according to our interpretations these 26 mice were the subjects of 41 primary tumors.

Our experience agrees with the observations of others that it is especially, and perhaps almost exclusively, the female mice which develop spontaneous tumors. In no instance has a male mouse possessing a spontaneous tumor come into our hands.

Our experience is further in agreement with previous observations which teach that by far the greater number of the spontaneous tumors of the mouse develop from the mammary glands. Of the 41 tumors which we have described, 29 arose in portions of the body corresponding with the distribution of the mammary glands.

Choosing the classification adopted by Apolant, we have found among the true tumors of the mammary glands representatives of each of his classes.

We also observed an example of an adenomatous tumor arising from a portion of the body corresponding with the distribution of the mammary gland which exhibited the structure of the typical sebaceous glands rather than the mammary gland, and which we have classified under the molluscoidal tumors of Haaland.

Metastases occurred in a certain number of the mice showing spontaneous tumors. When present they were exclusively in the lungs. We did not observe a single instance of metastasis to other organs, or to the lymphatic glands.

Next to the mammary glands the lungs showed in our series the most numerous primary tumors. According to our interpretations we encountered nine examples of primary pulmonary tumors, which were always associated with superficial tumors of the body. It is noteworthy that we have not come across a single instance of a primary pulmonary tumor among several thousand mice not showing superficial spontaneous tumors, which have been subjected to careful post-mortem examination. It would be easy to conclude, therefore, that the occurrence of spontaneous tumors in one part of the body in mice predisposes them to the development of spontaneous tumors of the lungs, but obviously such a deduction is not warranted at present. The first example of this type of tumor was described by Livingood.

Our observations of two primary sarcomata of the superficial tissues of the mouse is apparently unique. We believe that we excluded, probably, the possibility that the tumors were mixed sarcoma and carcinoma. Moreover, they exhibited properties unlike the superficial carcinoma of the mouse, since they were far more invasive locally. Our success in transplanting both of these sarcomata is also unique. In the first case a single successful graft which was not further transplanted was obtained; in the second case the sarcoma is being successfully transplanted at the present time.

We observed two examples of tumors developing from the lymphatic organs. One of these belonged obviously to the so-called lympho-sarcoma of mice. The other, we believe, is unique in so far

as its identification with the pathological condition described in human beings under the name of Hodgkin's disease is concerned. Furthermore, we succeeded for the first time, apparently, so far as the records in the literature go, in transplanting successfully a lympho-sarcoma. In one mouse an undoubted development of the transplanted graft occurred.

We also observed two examples of ovarian tumors, both of which were of the nature of papillary cyst-adenoma. These tumors arose in mice which were the subjects of spindle-cell sarcoma of the superficial tissues.

Our experience in the transplantation of the spontaneous mouse tumors agrees generally with that of other investigators. It would, however, appear that we have been on the whole more successful than others in the transplantation of the hemorrhagic class of mammary gland tumors, the so-called hemorrhagic cyst-adenomata. An explanation for this discrepancy cannot be readily given. It is, of course, possible, since such great variations are known to exist among mice as regards their susceptibility to tumor implantation, that the American mice are more subject to implantation of the hemorrhagic tumors than the European mice, but it is also possible that we have interpreted somewhat more broadly than others the class of hemorrhagic tumors. If the latter statement is true, the tendency was an unconscious one, and suggests that different workers will probably interpret these tumors quite differently.

Finally, this study indicates that mice are subject to a wide variety of spontaneous tumors, and suggests that the more widely the study is carried, the greater will be found the number and variety of tumors to which they are susceptible.

For the present we prefer to believe that spontaneous tumors are more frequent in female than in male mice, but that they also will be found to occur perhaps not very uncommonly among the latter. The point should be borne in mind that by far the greater number of spontaneous tumors of mice which have thus far come under observation have been present in old female mice. The business of breeding mice for sale leads to the retention of the female breeders until they become exhausted in this function by age, and the changing and weeding out of the males while they are still much younger.
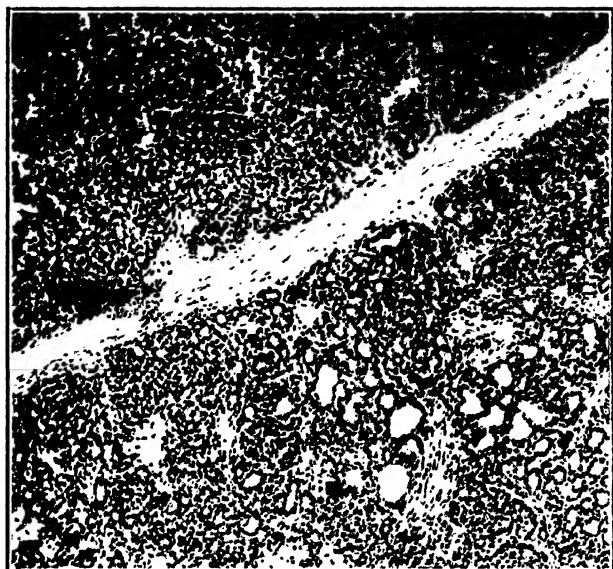
FIG. 1.
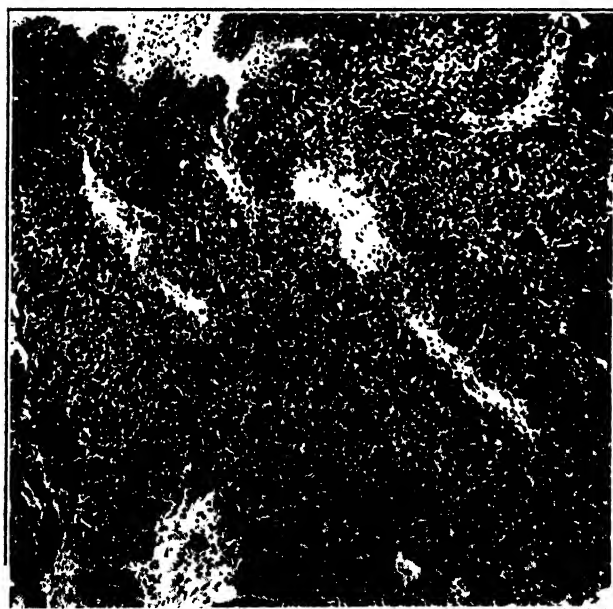
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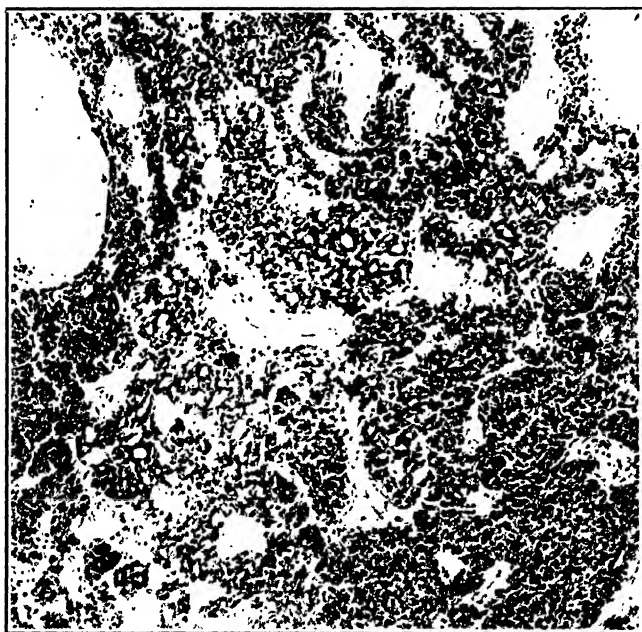
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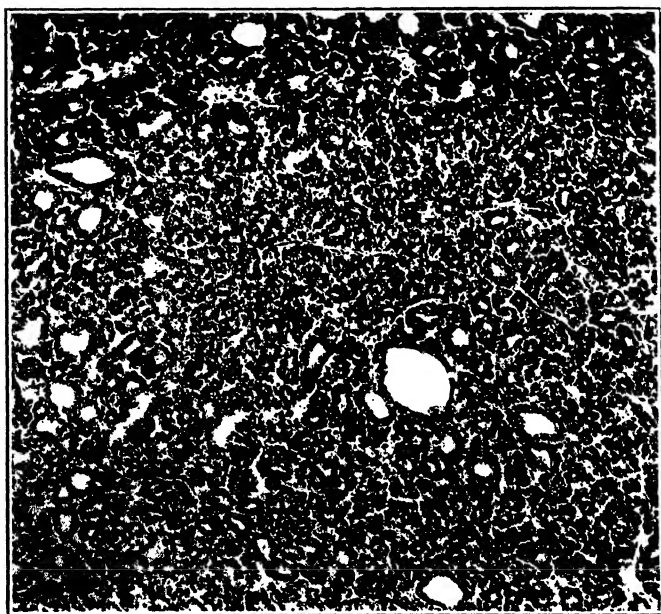
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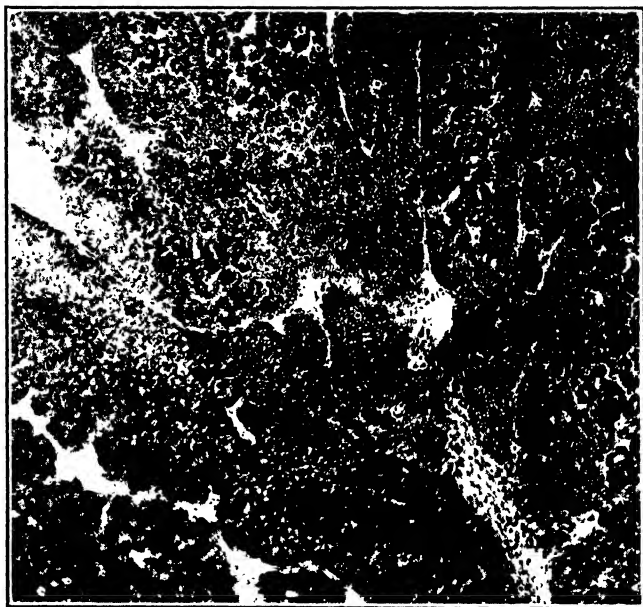
Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

FIG. 9.

FIG. 10.

FIG. II.
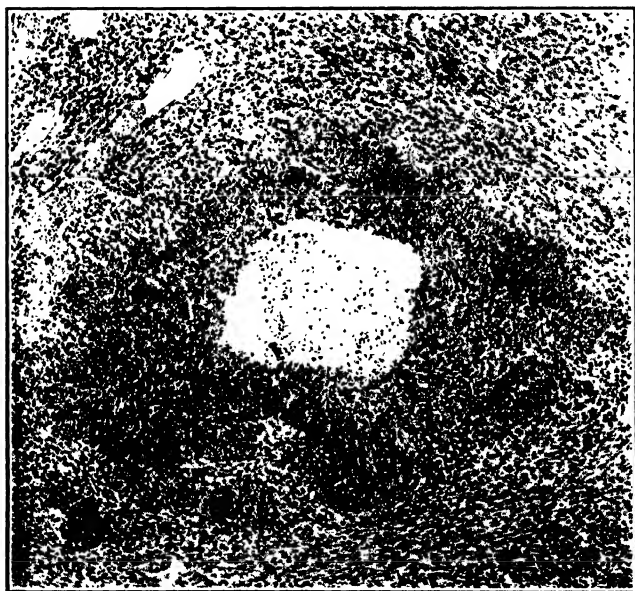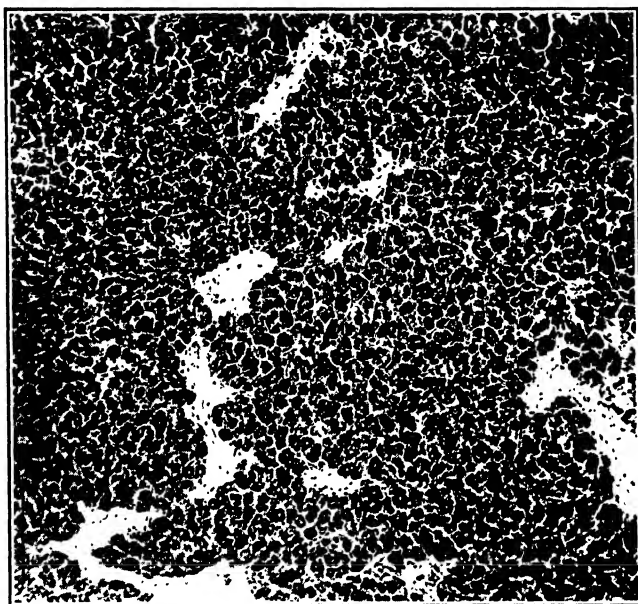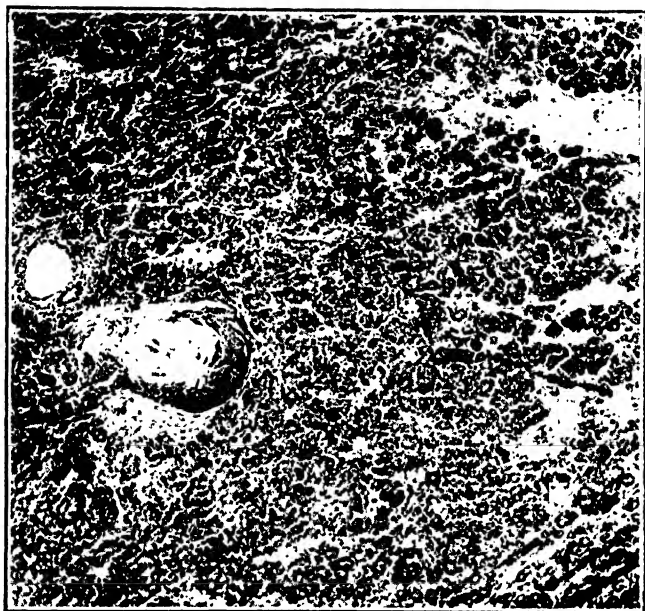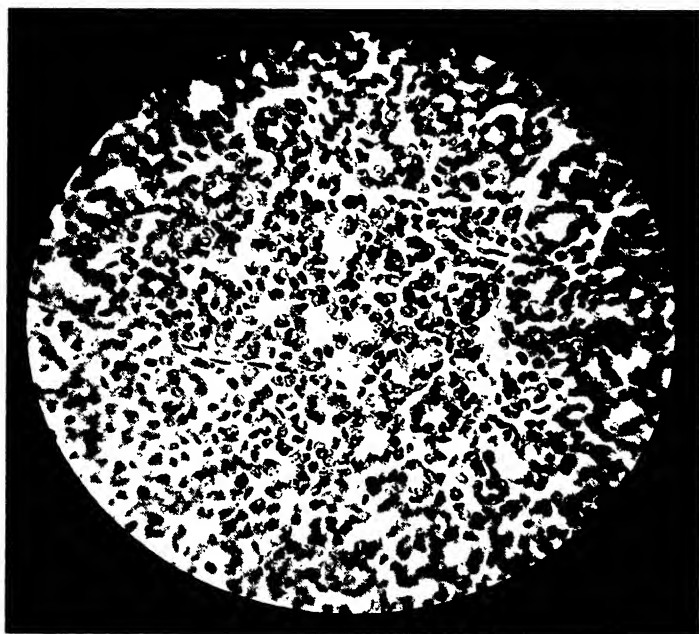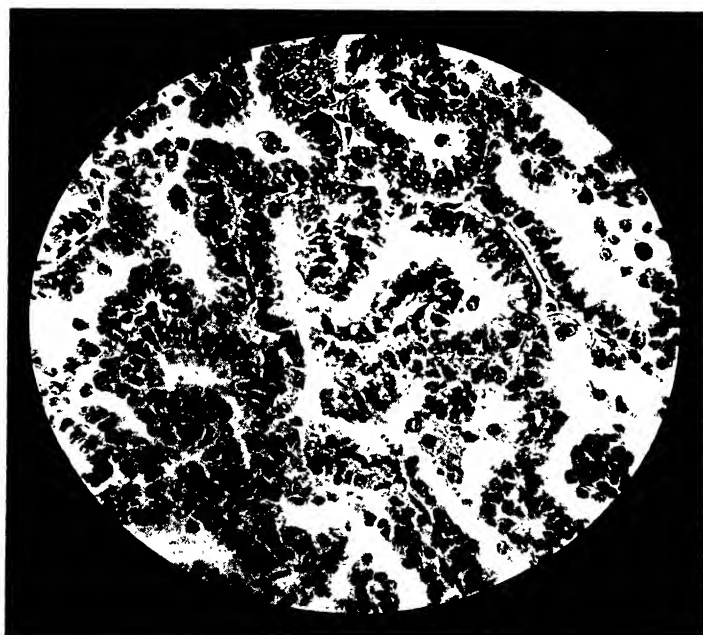
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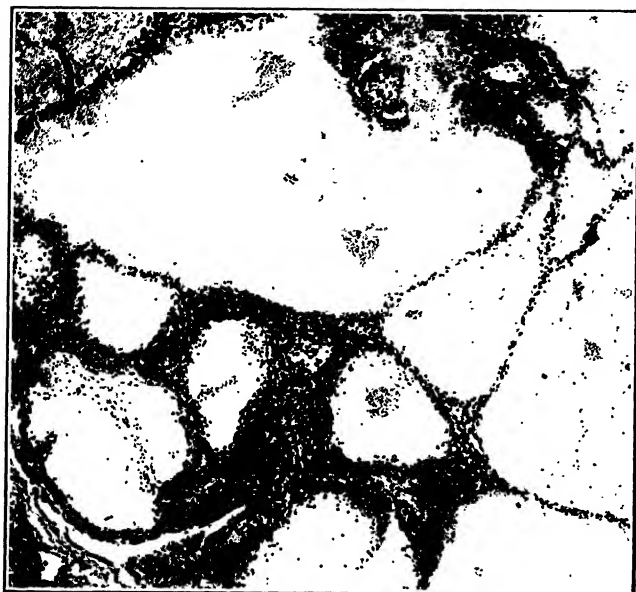
Fig. 13.

Fig. 14.

FIG. 15

FIG. 16.
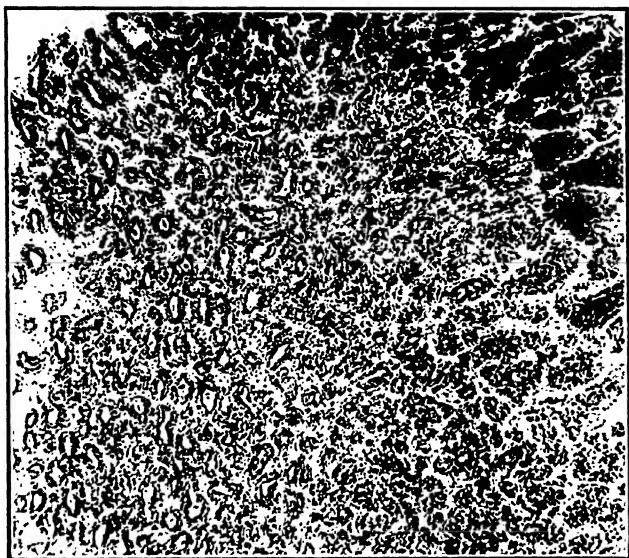
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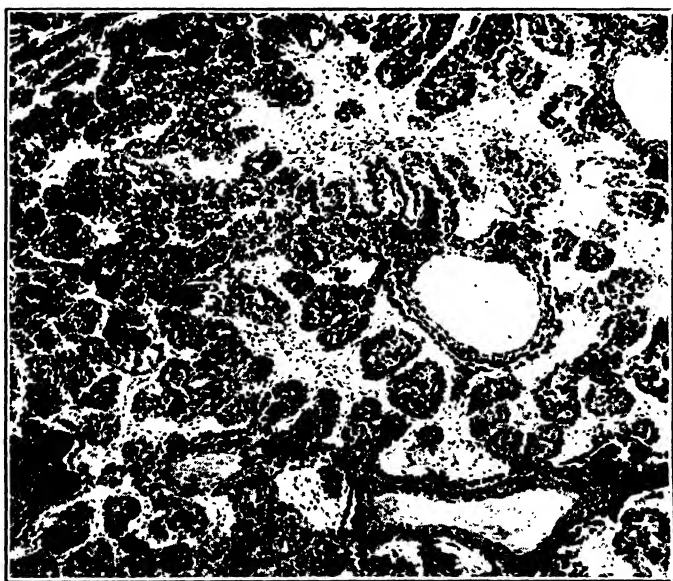
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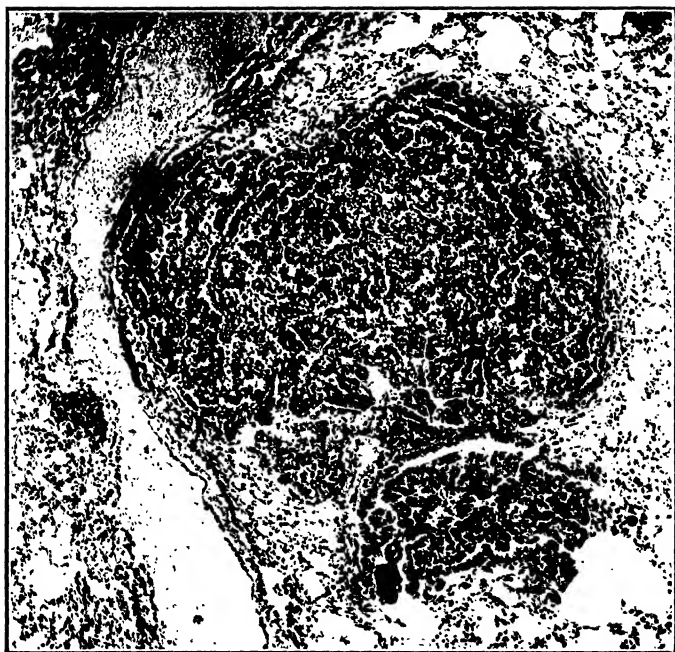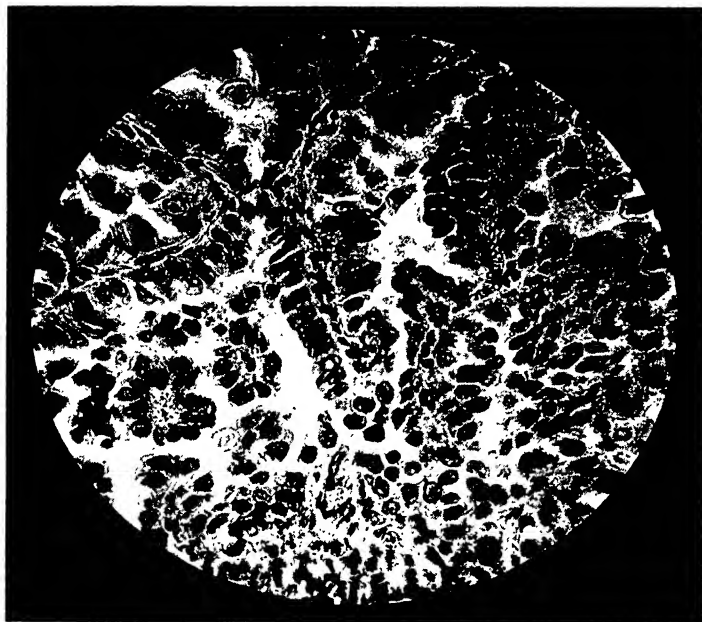
FIG. 20.
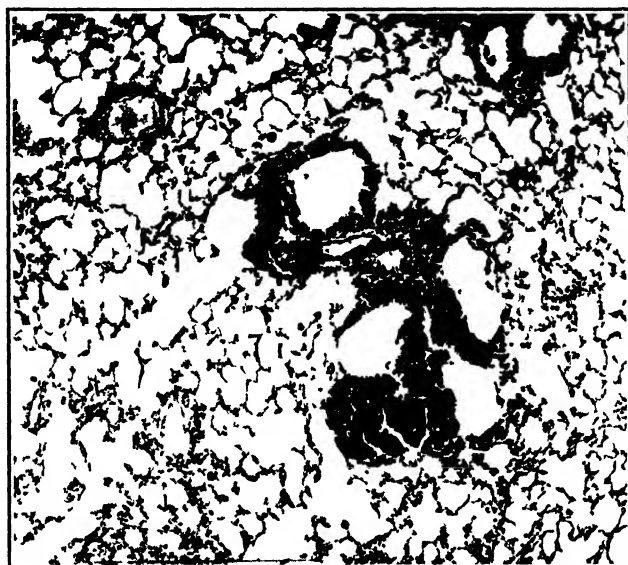
FIG 21
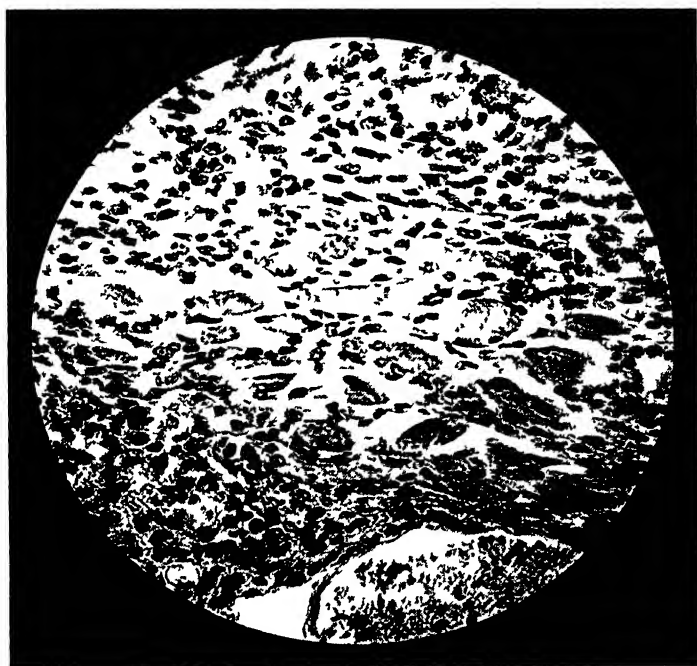
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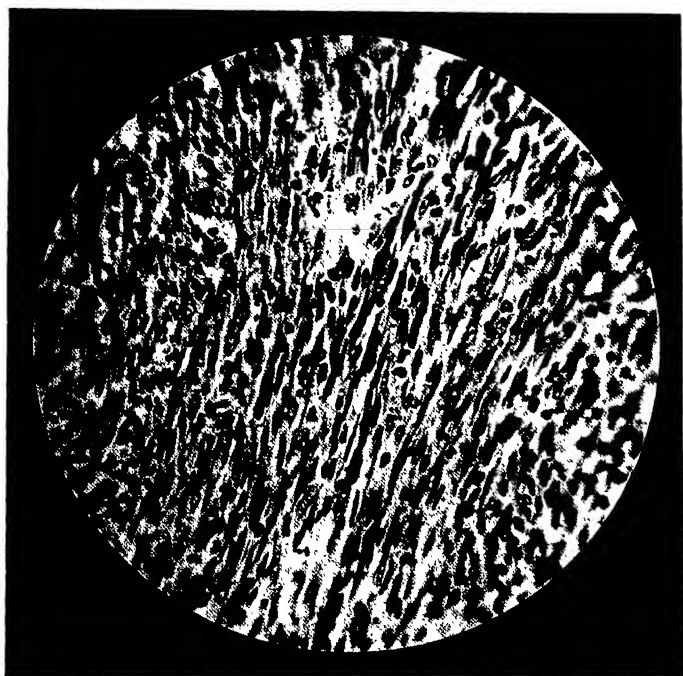
FIG. 23.

Consequently, far fewer males reach comparatively old age in the sense that the female breeders do, and this may be an important factor in accounting for the difference in susceptibility to spontaneous tumors hitherto observed in the two sexes.

EXPLANATION OF PLATES.

PLATE XVII.

FIG. 1. Original Mouse Tumor iv. Adeno-carcinoma. In the central and upper portions the growth is almost purely adenomatous, although there are also several nests of cells in which the glandular arrangement is not evident. The lower right hand portion shows the solid areas more clearly.

FIG. 2. Daughter tumor of fifth generation. The growth here is solid and there are no evidences of acini. The stroma is edematous. The presence of fat cells just below the center and to the left of it denotes the invasive character of the tumor.

PLATE XVIII.

FIG. 3. Daughter tumor of sixth generation. Shows apparent reversion to original type. It is composed almost wholly of acini and shows marked edema throughout. It is very similar to the primary tumor, though the stroma is not so well developed.

FIG. 4. Primary Tumor vi. The tumor is composed mainly of acini, some of which have become dilated and form small cysts. The stroma is not well developed. Solid nests of cells are not infrequent.

PLATE XIX.

FIG. 5. Daughter tumor of second generation. The growth is solid and there are no evidences of acini. The stroma is very delicate in character. A few blood-vessels with very thin walls are to be seen.

FIG. 6. Daughter tumor of the second generation. Lobules show necrotic centers. The stroma separating the lobules is well developed and very cellular. Numerous large blood-vessels with very thin walls are to be seen. The appearance here is very suggestive of a beginning sarcoma.

PLATE XX.

FIG. 7. Primary Tumor xiii. Adeno-carcinoma. The growth is composed of acini which are separated from one another by a very delicate stroma. Several blood-vessels are to be seen.

FIG. 8. Daughter tumor of third generation. The growth is composed of lobules, the central portions of which are necrotic. A few acini are to be seen between the lobules.

PLATE XXI.

FIG. 9. Primary Tumor xxiv. Adeno-carcinoma. This shows a small area of keratinization in the more solid portion of the tumor. Numerous acini are present.

Fig. 10. Primary Tumor xxv. The mass of keratin in the center is surrounded by numerous large cysts and a number of normal appearing acini. The cysts are filled with a finely granular and faintly staining material. In places the acini are widely separated by the serous exudate.

Plate XXII.

Fig. 11. Primary Tumor xxv. Extra vascular lung metastasis. There are numerous acini, although the growth is composed mainly of nests of cells. Several mitotic figures are present. The stroma is very delicate.

Fig. 12. Primary papillary cyst-adenoma from the lung of the same mouse. This figure is to be compared with Fig. 11. The former is a metastasis from the large subcutaneous tumor shown in Fig. 10. The irregular papillary ingrowths are covered by a single layer of columnar epithelium. A few necrotic cells are to be seen in the cavities. The connective tissue framework is not well developed. The different characters of the growths shown in Figs. 11 and 12 justify us, we think, in concluding that the tumors are entirely independent of one another in their origin.

Plate XXIII.

Fig. 13. Mouse Tumor iii. Daughter tumor of first generation. This shows very well the hemorrhagic type of adeno-carcinoma. There are numerous large cysts into which hemorrhages have occurred. Those cysts not showing hemorrhagic contents are usually filled with a serous exudate. The growth between the cysts is usually solid in character, though a few acini are to be seen.

Fig. 14. Mouse Tumor iii. Tumor of second generation. The growth shown here is to be compared with that of the first generation as shown in Fig. 13. The tendency here is towards the formation of irregular ·clefts and papillary ingrowths. This is the " spalten bildendes " type of Apolant. Hemorrhages are much less frequent in this tumor. The stroma is rather cellular and well developed in places.

Plate XXIV.

Fig. 15. Primary Tumor x. This is also typical of the hemorrhagic type of adeno-carcinoma. In the center are two large blood-vessels in the midst of necrotic tissue. The walls of the vessels are very thin and are surrounded with a thin mantle of tumor cells. Hemorrhages are to be seen everywhere throughout the section. Numerous acini are present.

Fig. 16. Mouse Tumor xx. Daughter tumor of first generation. This tumor is composed of lobules, the central portions of which are necrotic, but the remarkable feature of the growth is the cellular stroma separating the lobules. In places the appearance of this tumor is very suggestive of a mixture of sarcoma and carcinoma.

Plate XXV.

Fig. 17. Primary Tumor xxii. Molluscoidal type. This shows the radiating . columns of cells at the periphery. Some of the columns show a lumen on cross section. The next figure shows that a number also contain masses of keratin. The appearance presented in the deeper portions is that of an adenoma.

FIG. 18. Primary Tumor xxii. Portion of same tumor which shows masses of keratin. The keratin occurs not only in large masses but is also seen in longitudinal sections of the smaller tubules. On the right the branching of the tubules is very evident.

PLATE XXVI.

FIG. 19. Primary lung tumor in Tumor Mouse xix. The structure here is typical of most of the primary lung tumors of our series. The papillary in-growths are covered with a single layer of rather high columnar epithelium. The stroma is delicate in character. The cell outlines are distinct and they can easily be differentiated from one another.

FIG. 20. Original Mouse Tumor xiii. Primary lung tumor. This growth is more compact than any of the others of this series. To the left of the center there is a small bronchus, the lumen of which has been entirely occluded by the tumor growth.

PLATE XXVII.

FIG. 21. Original Mouse Tumor xiii. This shows what apparently is the point of origin of the primary lung tumor shown in Fig. 20. The growth was wedge-shaped and this corresponds with the apex which apparently was located at the termination of one of the bronchioles.

FIG. 22. Primary Tumor xviii. Sarcoma. The growth is composed of spindle-shaped cells which can be seen invading the surrounding muscle tissue. Some of the muscle cells are undergoing pressure atrophy, but the majority are well preserved.

PLATE XXVIII.

FIG. 23. Primary Tumor xxvi. Sarcoma. The cells are irregular in size and shape, but the dominant type is that of a spindle-celled sarcoma.

TRANSPLANTATION EXPERIMENTS IN MACACUS RHESUS WITH A CARCINOMATOUS TERATOMA FROM MAN

By J. W. JOBLING, M.D.

TRANSPLANTATION EXPERIMENTS IN MACACUS RHESUS WITH A CARCINOMATOUS TERATOMA FROM MAN.

BY J. W. JOBLING, M.D.

(From the Laboratories of the Rockefeller Institute for Medical Research, New York.)

Although many attempts have been made to graft human tumors upon the lower animals none have been really successful. The experiment of Roux and Metchnikoff[1] with anthropoids was a failure. The use of the dog, rat, etc., has not given promising results. The remarkable experience Lewin[2] relates of the development of a granulation tissue tumor in a dog inoculated from a malignant adeno-cystoma of the ovary has not yet been satisfactorily accounted for.

With our present knowledge gained chiefly from studies of transplantable tumors of mice, rats and dogs, we can predicate that even when transplantation is easily accomplished within species it fails when attempted between nearly related species. Hence we should not expect that fragments of tumors removed from human beings should develop to any extent in other species of animals. However, a final decision on this point may well be deferred until a number of essays with a wide variety of tumors and several species of animals have been made. At present we can view the problem of engrafting tumors upon heterologous species from two points: first, in relation to true grafting in which the tumor fragment actually takes hold and grows; and second, in relation to mere ability to increase somewhat in size for a short time, owing to temporarily favorable conditions of nutrition and restraint of autolytic disintegration. This indirect form of growth, which Ehrlich[3] first described under the

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[1] Roux and Metchnikoff, *Bull. de L'Acad. de méd.*, 1903, 1, 101.

[2] Lewin, *Zeit f. Krebsforsch.*, 1906, iv, 55.

[3] Ehrlich, P., *Arb. a. d. k. Inst. f. exper. Therapie*, 1906, No. 1, 2.

name of zig-zag transplantation, does not always occur, and probably is dependent somewhat upon nearness of relationship between the species—one that supplies and one that receives the grafts. That some such factor operates at times is rendered probable by the far greater reactions of inflammation which occur when the species are widely separated than when nearly related. Dr. Flexner and I have made observations of this kind with a transplantable rat tumor ingrafted in mice, rabbits, and guinea-pigs. A similar interpretation can be put upon some of Loeb's[4] experiments.

It is now generally recognized that in order to secure satisfactory results the transplantations must be carried out with great care so as to avoid bacterial infection and consequent inflammatory reaction, and no foreign fluids should be allowed to come into contact with the graft. Unless these precautions are observed failure may result even with readily transplantable tumors, and when the fate of the grafts is to be followed day by day by accurate measurements, the inflammatory reactions about the fragments must be controlled. From this source alone actual increase in size may occur and it can be excluded as a factor only by excising some of the enlarged grafts and subjecting them to microscopical examination.

The history of the tumor, which follows in brief form, is convincing as to its malignancy.

S. L., female, white, age 14 years. Patient was first admitted to the Presbyterian Hospital in the service of Dr. A. J. McCosh on January 18, 1908. One sister when sixteen years of age had a dermoid cyst removed. Patient had an enlargement of the abdomen which had been present for two months. A diagnosis of a dermoid cyst of the ovary complicated by intestinal obstruction was made. The patient was operated upon the day of admission. There was found a smooth walled cyst the size of a foot-ball, which was friable and contained a mass of heterogeneous tissue. The cyst was first drained and then drawn into the incision, when a large amount of grumous gelatinous material escaped.

Pathological Report.—(Kindly supplied by Dr. Opie.) The tumor is made up of fibrous tissue, some striated muscle, and a number of hair follicles. There are also some papillomatous structures and epithelial whorls.

The patient appeared relieved until twelve days after the operation, when pain, vomiting and distention of the abdomen appeared, and with these symptoms a perceptible mass in the original locality. The mass continued to develop until February 9, when it was again operated upon. At the operation there

[4] Loeb, L., *Jour. of Med. Research,* 1902, viii, 1.

was found a mass of old organized and inorganized blood in the region of the hepatic flexure of the colon and considerable fibrin and masses of tissue looking like cyst elements (bone, cartilage, etc.) in the lower portion of the abdomen. The report from the pathological laboratory on the material obtained at this operation was "that it consists of masses of hyaline cartilage which are infiltrated with a few polymorphonuclear cells. The stroma is composed of fibrous tissue, fat and extravasated blood, and contains numerous cells of the polymorphonuclear and lymphoid type."

The patient was discharged three weeks later apparently well, but after another three weeks returned on account of a growth about the size of a cocoanut in the original locality. She was operated upon the following day, when there was found a mass filling the right lower quadrant of the pelvis, which was composed of a tenacious, hemorrhagic material involving all the structures in this locality and having no apparent point of origin. The serosa of the intestine contained a number of miliary nodules. It was with a portion of the material removed at this operation that the first transplantation experiments were conducted.

The material which was used for inoculation consisted of a soft, friable mass of the consistency of brain tissue showing hemorrhagic spots.

Microscopically the tissue consisted of hyaline cartilage, glandular columnar epithelium and a cellular stroma. The stroma is rich in cells and in places edematous. The cells are spindle-shaped or polygonal with rather large, pale vesicular nuclei. · In places the stroma is finely granular and contains numerous fine vacuoles, this appearance probably being due to edema. It is exceptional to find a definitely fibrillated area, the tissue probably being too young for this. Not infrequently one finds a small clump of embryonic striated muscle cells in the stroma. Small hemorrhages are present. Mitotic figures are fairly numerous. Imbedded in this stroma are masses of hyaline cartilage. These masses are usually small and almost round in shape, though in some instances they are larger and more irregular in form. They are scattered throughout the sections and do not appear to bear any definite relation to either stroma or clumps of epithelial cells. They are very cellular and many of the cells are undergoing mitosis. Some of the masses are sharply differentiated from the surrounding stroma by a layer of closely packed cells, the nuclei of which are oval or elongated, with the long axis pointing either toward, or parallel with the periphery. Mitotic figures are present in the central as well as in the peripheral portions.

The epithelial type of cell is present everywhere, either in the form of irregular clumps without any definite arrangement, or in the form of an acinar or gland-like arrangement lining irregular clefts and tubules. In most instances the cells lining the tubules or clefts are arranged in several layers, though, exceptionally, places are seen in which the tubules are lined by a single layer. Goblet cells are not infrequent among those lining the tubules. The cells are usually large, with vesicular nuclei, though nuclei rich in chromatin are not infrequently seen. Without having regard to the arrangement of the cells, mitotic figures are very numerous.

Owing to the rapidity of the growth after recurrence of the tumor, Dr. McCosh offered it for inoculation experiments. We obtained the tumor immediately after its excision, wrapped in sterile dressings to prevent infection and evaporation, and made the implantations into macacus monkeys, rats and mice. The technic used was that followed in the transplantation of rat and mouse tumors. The tumor was cut into small fragments, no fluid of any kind being used for the subcutaneous inoculations, and one of the fragments placed in the distal end of a hollow needle. After cutting away the hair over the site of inoculation, the surface was cleansed with alcohol and the alcohol removed with sterile gauze. The needle was then introduced into the subcutaneous tissues and by means of a sterile platinum wire, just fitting the lumen, the fragment was pushed out of the needle into the tissues beyond. The inoculations were always made beneath the skin of the abdomen. Every precaution was taken to avoid infection. The time elapsing between the removal of the tumor from the patient and its inoculation into the animals was about forty-five minutes, the chief loss of time arising from the distance between the Presbyterian Hospital and the Rockefeller Institute.

Monkey 1.—This animal was inoculated in eight places with the tumor fragments. In four places it received tumor fragments alone; in two places, tumor fragments plus a drop of a saturated solution of scharlach R. in olive oil; in two places, tumor fragments plus a drop of a saturated solution of sudan III in olive oil; in one place, scharlach R. solution alone; and in one place sudan III solution alone.

7th day. There are distinct masses to be felt at the points where the fragments were lodged. These measured from 2 to 3 mm. in diameter.

9th day. Fragments about the same size. At this time the larger portion of two of the fragments were excised. The fragments to which the scharlach R. and sudan III solutions had been added at time of inoculation do not appear as large as those inserted without any chemicals. The material excised was used to inoculate two other monkeys.

11th day. The nodules not operated upon remain about the same size. Considerable swelling at sites of operation.

13th day. The nodules are somewhat smaller than at previous examinations.

16th day. Excised one of the nodules for histological study. Fragment removed measured about 2 mm. in diameter.

18th day. Fragments smaller, but still perceptible.

20th day. Fragments just perceptible.

22d day. Fragments disappeared.

The material excised from Monkey 1 was used to inoculate Monkeys 2 and 3.

Monkey 2. This monkey was inoculated in five places with material excised from Monkey 1.

4th day. All the fragments are easily felt and measure 2 mm. in diameter.

5th day. Excised fragment for histological study. The fragments measure 3 mm.

7th day. No change in size.

11th day. Fragments somewhat larger, measure 4 mm.

15th day. Fragments measure about 3 mm.

19th day. Fragments smaller.

21st day. Fragments disappeared.

Monkey 3.—The animal was inoculated in five places with material excised from Monkey 1.

4th day. Fragments measure 2 mm. in diameter.

7th day. One of the fragments remains about the same size, the others somewhat smaller.

12th day. Fragments somewhat larger and measure 4 mm.

15th day. Fragments disappeared.

As stated in the protocols, several of the fragments were removed for histological examination.

The first fragment examined was excised from Monkey 1 on the eleventh day after inoculation. The sections show a moderate amount of connective tissue separating the islands of cartilage which are several times the size of the islands in the transplantable tissue. The epithelial cells have all disappeared and the stroma of the transplanted fragment is much less cellular than in the original tumor, there are more connective tissue fibrils and the cells of the stroma are more elongated than in the original tumor. In other words, the tissue which surrounds the islands of cartilage is a reaction product chiefly, although part of it is possibly derived from the old

stroma. The cartilage appears normal, has not been invaded with connective tissue, and a small number of the cells are in active mitosis.

The second fragment examined was also obtained from Monkey 1, but was excised on the sixteenth day. Sections from this fragment show that there has been no marked change in the masses of cartilage, but the intervening connective tissue between the small masses is much more cellular. Many of the cells in the connective tissue are oval and resemble the stroma cells of the original tumor, and the amount of fibrillated tissue is less than in the fragment removed on the eleventh day. A few mitotic figures are still present.

The next fragment examined was the one removed from Monkey 2. This fragment had been nine days in Monkey 1 and five days in Monkey 2, or in the two monkeys for fourteen days. Microscopically the fragment is seen to be encapsulated with fibrous tissue, which in the center is quite hyaline. The cartilage masses are degenerating, (1) through loss of nuclei chiefly in the center and by degeneration of nuclei at the periphery, and (2) by a diffuse blue (hematoxylin) staining in the center of some of the masses, probably due to a calcareous deposit. In this situation the cartilage cells are shrunken, clear spaces exist in the matrix about them, and the nuclei are deformed and pyknotic. In the periphery of some of the cartilage masses mitosis is still to be seen.

The next fragment examined was also from Monkey 2. This fragment had been for nine days in Monkey 1, and for twenty days in Monkey 2, or twenty-nine days in both animals.

Microscopically the sections are composed almost entirely of fibrous and adipose tissue, and accumulations of small (lymphoid) cells and numerous eosinophiles. Nothing remains of the tissue transplanted.

Following the operation of March 23 the patient became progressively worse, the growth of the tumor being even more rapid than before, and she died on May 4, 1908. At each dressing of the operation wound a portion of the mass would adhere to the gauze or could be easily. detached with forceps, and with one such piece another transplantation experiment was made.

Histologically the main difference between the material received at this time and that at the preceding operation consists in this: the growth now contains no cartilage and is composed of the cellular stroma and masses of epithelial cells often appearing as acini. Mitoses were numerous in both stroma and epithelium.

Monkey 4. This animal had not previously been inoculated. It received six fragments subcutaneously.

4th day. Fragments cannot be felt.

8th day. Distinctly visible. Measure 2 to 3 mm. in diameter.

12th day. Fragments disappeared.

Monkey 5. This was also a new animal. It received four fragments sub-cutaneously, and 2 c.c. of an emulsion, made by teasing the tumor in sterile ascitic fluid, was injected into the peritoneal cavity.

4th day. All fragments show apparent growth and measure 2 to 3 mm.

5th day. Removed one fragment for histological study.

8th day. Fragments measure about 3 mm.

12th day. Barely perceptible.

14th day. Disappeared.

Monkey 2. This animal had previously been inoculated with fragments from Monkey 1. These fragments had shown a marked increase in size, but at the time of reinoculation, on the fifteenth day, had already begun to be absorbed and measured about 3 mm. The monkey was now reinoculated in four places with new tumor material.

4th day. The fragments from first inoculation measure 2 mm. in diameter, those from the recent inoculation measure 2 to 7 mm.

5th day. One of the new fragments measuring 7 mm. was excised. A portion was used to inoculate monkey 6 in six places, and the remainder was saved for study. The fragment excised was rather sharply circumscribed and firm in consistence. Complete disappearance of fragments.

8th day. New fragments measure 7 mm.

12th day. Fragments measure 3 mm.

14th day. Barely perceptible.

20th day. Disappeared.

Monkey 3.—This animal had also been previously inoculated with a fragment excised from Monkey 1. These fragments had shown a marked increase in size, but at the time of reinoculation on the fifteenth day had undergone complete absorption.

It was reinoculated in four places with the new material.

4th day. The fragments measure from 4 to 7 mm.

8th day. Measure 6 to 7 mm.

12th day. Measure 3 mm.

14th day. Measure 3 mm.

20th day. Disappeared.

Monkey 6.—This was a new animal and was inoculated in six places with the fragment excised on the fifth day from Monkey 2.

The animal died two days later from an intercurrent disease. The fragments, which appeared pale, were saved for sections.

Some of the tumor material used to inoculate the monkeys in this second experiment was also used to inoculate five rats and five mice. These animals were examined every second day for some time, but at no examination was there any evidence of the fragments having increased in size.

Microscopical examination of the fragments excised on the fifth day from Monkey 5 shows that it consists of fibrous tissue enclosing masses of small cells (lymphocytes) and some leucocytes. No epithelial cells or cartilage are present. The increase in size of the fragment was caused in this case by inflammatory reaction.

Examination of the fragment removed on the fifth day from Monkey 2, which was used to inoculate Monkey 6, showed practically the same conditions noted above. The eosinophilic cells were more numerous than in other instances. The fragments removed from Monkey 6, which died two days after inoculation, were all necrotic.

From the experiments recorded, it is obvious that for a time the grafts increase in size under the skin of the monkey and do not increase under the skin of mice or rats. The microscopical appearance of the excised fragments indicates clearly what the causes of the increase in size are. It was found, in the first series of experiments, that the inflammatory reaction about the graft had been comparatively slight, that the epithelial elements had not survived but had entirely disappeared, and that the cartilage had not only remained alive for sixteen days, but still showed mitosis at the end of that period and had increased several fold in size as compared with the largest islands contained in the original tissue. Examination of sections made from the tumor material used in the second series of experiments shows that no cartilage was present and in the masses removed from the site of inoculation the epithelial cells and stroma of the original graft have been replaced by inflammatory tissue. As stated above, two series of experiments were conducted. In the first series only one monkey was inoculated with the nodules developing in this one, and two others were inoculated in six places each.

In the second experiment, with fresh tumor material, two new

monkeys were inoculated, and at the same time two of the old monkeys were inoculated.

Reference to the detailed description given for each monkey shows that in the three monkeys of the first series of experiments the measurements of fragments gave almost exactly similar figures on the corresponding days, but the examination of the second series in which new tumor material was used shows that the same relations do not exist.

These points are very well shown in the accompanying table.

Day.	Approximate size of piece injected.	First series.			Second series.				
		Monkey 1.	Monkey 2.	Monkey 3.	Monkey 4.	Monkey 5.	Monkey 2.	Monkey 3.	Monkey 6.
4	0.5 mm.		2	2	0	2	4.5	5.5	0
5	"		3						
7	"	2	3	2.5					
8	"				3	3	7	7	
9	"	2.5							
11	"	3	4	4	0	1	3	3	
14	"	3	3	0	0	0	1	3	
16	"	2	2	0	0	0	0	0	
18	"	1	1	0	0	0	0	0	
20		?	0	0					

There were four monkeys in the second series; two had never been inoculated and two had been used in the first set of experiments. Reference to the measurements obtained with the new monkeys shows that there was progressive increase in size of the fragments up to the eighth day, but that following this there was a rapid retrogression leading to complete absorption. In the two monkeys which were reinoculated, the increase in size was about twice that noted for the fragments in the other two monkeys of this series. The maximum size was reached on the eighth day, following which there was a progressive diminution in size, though in one monkey on the fourteenth day the fragment measured three millimeters. Complete disappearance was first noted on the sixteenth day. The differences noted in this series between the monkeys which were inoculated for the first time and those which were reinoculated are probably due to the greater reaction in the subcutaneous tissues of the latter, corresponding perhaps to the reac-

tion first reported by Arthus[5] which occurs in the subcutaneous tissues of rabbits following a third or fourth injection of horse serum.

Although the attempts to implant the tumor fragments obtained from a human subject upon monkeys failed, yet it can be said that certain of the fragments increased considerably—three or four times the size of the original fragments—in the subcutaneous tissues of the monkey and the increase continued up to the sixteenth day after the transplantation. During that period organic connection of the transplanted tissue with the monkey had taken place by means of a granulation tissue which had surrounded and invaded the fragments. It was found that all the teratomatous elements of the tumor did not survive or grow, but that only one element, the cartilage, was able to survive and increase in size. At the end of eleven days the epithelial cells had entirely disappeared and no trace of them could be found. It is possible that some of the cellular stroma of the original tumor persisted and formed part of the enveloping stroma of the cartilaginous masses. The oval cells of the stroma of the original tumor are indistinguishable from the oval cells of the transplanted fragments. That the cartilage of the implanted fragments has remained alive is shown by the excellent condition of its cells and the absence of leucocytic invasion, and that it has actually grown is indicated by the mitoses which are almost as numerous in the specimen secured sixteen days after implantation as in the original tumor, and by the obvious increase in the size of the cartilaginous masses. Thus it has been found that cartilaginous tumor tissue from men can remain alive and proliferating for sixteen days at least when implanted beneath the skin of certain monkeys, while epithelial cells, undergoing active mitosis, derived from the same tumor, quickly die and disappear.

The fact is worth noting that the results of the implantation of teratoma of human origin have been wholly different and entirely negative in certain lower animals—rats and mice—which are further removed in respect to evolution from man than is the monkey. I would, therefore, point out that it is not permissible to conclude from one set of experiments on widely removed species

Arthus, *Compt. rend. Soc. de biol.,* 1903, v, 817.

that tumor implantations between heterologous species is absolutely impossible.

Finally, I am of the opinion that the results which we have obtained in the experiments on monkeys are not to be interpreted as an actual partial success in transplanting tumors from man to the monkey, but rather as an illustration, among the highest vertebrates, of the existence of a certain kind of imperfect obstacle to tumor growth between species, which has been expressed by Ehrlich[6] in his conception of atreptic immunity. In other words, the tumor cells introduced into the body of the foreign species are not at once wholly destroyed by the fluids and cells of the host, but are gradually exhausted by the want of a special and peculiar nutriment which the foreign host cannot supply, although a sufficient quantity is carried along at the time of inoculation to enable the foreign tumor cells to survive for a period. On the exhaustion of this peculiar nutritious element, the transplanted cells undergo a form of slow starvation retrogression.

[6] Ehrlich, P., *Arb. a. d. k. Inst. f. exper. Therapie,* 1906, No. 1, 84.

ON THE EXPERIMENTAL SURGERY OF THE THORACIC AORTA AND THE HEART.*

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INTRODUCTION.

DURING the last winter, I performed some experiments with the view of improving the general technic of intrathoracic operations and of finding special methods which could be used for the treatment of certain diseases of the heart and of the aorta. It is probable that the aneurisms of the thoracic aorta could be extirpated and the circulation re-established by a vascular transplantation, if a proper technic was developed. It seems possible, also, that some valvular and vascular diseases of the heart might be improved by surgical therapeutics. Nevertheless, the surgery of the heart has been limited entirely to the treatment of the wounds. A few experimental studies of this subject, however, have been made in the laboratories of Frederick in Marburg and of Harvey Cushing in Baltimore. But, in spite of their comparative simplicity, these experiments have been followed by a heavy mortality. Their results and the high death rate given by the operations on human beings show that the general technic of intrathoracic surgery is still insufficiently developed. Therefore, before describing the special methods that I found, it is necessary to study the procedures which permit the use of them without great danger.

GENERAL TECHNIC.

The bad results following the intrathoracic operations in experimental as well as in clinical surgery are due to a lack of adaptation of the technic to the physiological conditions of

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* Read before the American Surgical Association, May 5, 1910.

the chest. The complications which often kill the animal or the patient are brought about directly or indirectly by the infection of the pleural or pericardiac cavities, or by the respiratory disorders caused by the penetration of the air into the thorax.

Pleural infection is the more dangerous of these complications. In one hundred fatal cases of wounds of the heart treated by suture, death was the result of sepsis sixty times. The constant failure of the extirpation of œsophageal cancer is due generally to the same cause. The experimental results are also very far from being satisfactory. Many animals operated by Sauerbruch and Haecker in Germany, or by Bernheim, Robinson, Janeway, and Green in this country died of purulent or serofibrinous pleuresis, or of pneumothorax caused by the secondary opening of a bronchus or of the thoracic wall. Nevertheless, the operations were generally not very complicated and extensive. The high death rate is due to the lack of understanding by many surgeons that pleura and pericardium do not react against infection as peritoneum does. The technic which permits a successful abdominal operation may be insufficient when used in intrathoracic surgery. The degree of infection which does not interfere with the healing *per primam intentionem* of a wound can probably cause the failure of certain vascular or thoracic operations. It is well known that between absolute asepsis and the degree of infection which produces the ordinary symptoms of inflammation there is a number of intermediate stages. The so-called aseptic wounds are almost always slightly infected. Often surgical asepsis is merely a condition of non-suppurative infection. But the attention of the surgeons has not been attracted by the various forms of attenuated infection, because it does not prevent the healing of ordinary wounds. Nevertheless it seems probable that the more marked states of non-suppurative infection can be very dangerous in intrathoracic surgery. It is, therefore, necessary to use in vascular and intrapleural operations better asepsis than it exists in many hospitals and laboratories.

This higher degree of asepsis can be obtained by the rigid application of principles known by every surgeon, but often neglected partially in the management of the operating rooms, the preparation of the patient, of the operators, the handling of the tissues, etc. The success of the more complex intra-thoracic operations depends on the observance of a number of minute details of technic. It is necessary mainly to remove some of the principal factors of irritation and inflammation of the pleuræ during the operation. The handling with forceps or retractors, the sponging, or the walling off with gauze, the exposition of large surfaces to the air bring about irritation of the pleura and facilitate greatly its infection. As soon as the thoracic cavity is opened, the lungs must be covered with fine Japanese silk compresses, impregnated with vaseline. The silk tissue acts as a thin and almost impermeable membrane which protects the pleura, without irritating it, against the contact of the fingers, and permits a very efficient walling off of the operating field. At the same time, it prevents the evaporation and the desiccation of the surface. In order to prevent the cooling of the viscera, a piece of thick flannel is placed on the silk compresses. Besides, the temperature of the operating is very high, 29 or 30° C. It is important not to allow the blood to flow through the pleural cavity. The sponging of the pleural cul-de-sac may be a cause of infection. All handling of the unprotected pleura or pericardium is dangerous.

The usefulness of a rigid asepsis is shown by the results which were obtained. I made twelve operations similar to those performed by previous experimenters, resection of pulmonary lobes, resection of a small segment of the middle part of the œsophagus, simultaneous opening of both pleuræ and pericardium, dissection of mediastinum and ascending aorta. It must be remembered that the suture of the œsophagus gave such constant mortality that Janeway and Green thought success was not possible and devised a very ingenious and complicated method for œsophageal anastomoses. Again, Sauerbruch, Robinson and others found that secondary pneu-

mothorax may follow lung resections, and Willy Meyer developed a very elaborate method for closing the bronchial stump. Nevertheless, I did not use those technics. I employed only the simplest sutures or ligatures, and the animals, without exception, recovered. Although this technic has given better results than those obtained by previous experimenters, it is still far from being ideal. After more complicated operations like patching of the vena cava, graft of vessels on the heart, simultaneous operations on the heart and the descending aorta, when very large exposure of the thoracic cavity is rendered necessary, I observed some serofibrinous or purulent pleureses. On twenty-eight operations of 'that class, seven times this complication occurred. Several animals died of secondary hemorrhage from aortic patches or cardiac sutures, and it is possible that the infection played also a rôle in these complications. Nevertheless there is no doubt that even in very complex operations, septic complications can be avoided. It is of great importance for the future of intrathoracic surgery that this part of the technic is completely developed.

The asphyxia which follows the opening of the thoracic cavity may be due to the pneumothorax, to a spontaneous stopping of the respiration, or to the inefficiency of the respiratory movements produced by a large opening or a strong retraction of the thorax.

The asphyxia produced by pneumothorax is easily prevented by the use of a Sauerbruch or Brauer chamber. Recently Willy Meyer and his brother Julius Meyer have built a splendid apparatus, which combines the advantages of both methods. On man, unilateral pneumothorax is not very dangerous and extensive intrathoracic operations have been performed without any apparatus. But it is very much safer to use a positive or negative pressure apparatus, which prevents completely the respiratory troubles caused by the pneumothorax.

When asphyxia is produced by the spontaneous stopping of the respiration, the Sauerbruch method becomes inefficient,

and does not prevent the patient or the animal from dying.   In the more extensive operations, when the chest is widely opened, and the thorax dislocated by a strong retraction, the ribs and the diaphragm cannot act any longer on the lungs, and asphyxia occurs.   In these cases also the Sauerbruch method cannot prevent the death of the animal.   On the contrary the life of the animal will go on normally, if the Meltzer and Auer method of intratracheal insufflation is used.   Their method has the very important advantage over all the others of permitting the respiratory exchanges to continue even if the respiratory movements have stopped or become inefficient.   The apparatus I used is composed of only a foot-bellows, a rubber tubing connected with an ether bottle, and a manometer, and a small intratracheal catheter.   With the method of Meltzer and Auer, the spontaneous respiration becomes a luxury because even when the respiratory movements cease or become inefficient, the ventilation of the lungs still goes on and the animal remains in excellent condition.   By its simplicity and its efficiency, the method of Meltzer and Auer is a great advance in experimental surgery.   It is actually the safest method for performing extensive operations on the chest.

Among the factors which bring about the success or the failure of an intrathoracic operation, the more important is not the apparatus which prevents the respiratory disorders caused by the pneumothorax.   The main danger is not asphyxia, but infection, as it has been demonstrated by the results of many experimental and clinical operations in this country and in Europe.   The technic must be improved, therefore, by better asepsis rather than by complicated apparatuses.

SPECIAL TECHNIC.

I attempted to find new methods which could be used for the treatment of the aneurisms of the thoracic aorta, and of the valvular and vascular diseases of the heart.

The diagnosis of the aneurisms of the aorta can be made to-day at a comparatively early period.   It is rational to think that some of them can be extirpated.   Therefore we must find

methods which permit repair to the aortic wall after a partial or complete resection.

We know that the lumen of an artery can be reduced without interruption of the circulation by the operation I described a few years ago as longitudinal exclusion. This method could be used for a sacciform aneurism, with narrow pedicle. But, in most cases, a resection of a part of the wall or of a complete segment would be necessary. I have already demonstrated that after a partial resection, an arterial wall can be patched with a piece of artery, of vein, or even with a piece of peritoneum. These operations present very little danger, and their results, observed many months after the graft, were excellent. I succeeded also in substituting to a part of the abdominal aorta a piece of rubber sheeting. The circulation went on normally and is still normal after several months. In a number of other operations, I found that entire segments of veins and of arteries, fresh or preserved in cold storage, can be transplanted on arteries. The results observed after several years are excellent, and the operation performed under certain conditions is very safe.

The patching and grafting of entire vascular segments would permit the re-establishment of a normal circulation after resection of a sacciform or fusiform aneurism. But, the technic must be adapted to the special anatomical and physiological conditions of the intrathoracic aorta. Its wall is exceedingly friable, especially in the ascending part of the arch. It is very easily cut by the threads. It has, therefore, been necessary to modify in a large measure the ordinary technic of vascular suture. The sutures and anastomoses on the thoracic aorta are less difficult but more dangerous than on the other arteries. Every detail of the technic must be directed toward the prevention of secondary hemorrhage. This complication is liable to occur after aortic suture, while it does never happen after suture of the abdominal aorta and other arteries. Parietal thrombosis, which is generally a very dangerous complication, does not produce any trouble when it develops on the aortic wall. On account of a possible

hemorrhage it is more dangerous to graft a piece of vessel on the thoracic aorta than on another artery.

It was necessary also to find out a method for diverting the blood during the operation, because the aortic circulation cannot be interrupted for a long time without the occurrence of nervous complications. The main danger of the aortic operation does not come from the heart or from the aorta itself, but from the central nervous system. When the descending aorta is clamped for more than ten or fifteen minutes, the posterior limbs become paralyzed. It is generally a spastic paralysis. The lesions are localized to the cells of the anterior horn. It seems that the clamping of the ascending aorta cannot be continued without danger for more than one minute. A smaller operation like the suture of a wound can be performed in less than one minute. We can also make a circular suture of the descending aorta in six and even in three minutes. But the resection of an aneurism, and the graft of a vessel would take a very much longer time. Therefore, I tried to develop a technic for the temporary diversion of the blood.

The diversion of the blood can be produced in two different ways—by temporary intubation of the aorta or by an artificial collateral circulation, *i.e.*, a *central* or *lateral* diversion of the blood.

The *central* diversion consists of tubing the segment of the aorta which is to be resected. The vessel is laid open by a longitudinal incision and a paraffined tube is inserted into its lumen and temporarily fastened. This small operation involves only a short interruption of the circulation. Then the wall of the aorta can be extirpated and replaced while the circulation goes on through the tube. When the operation is completed, the tube is removed through a small incision in the wall of the aorta. There is no danger of coagulation of the blood in the tube during the operation. The patching or the transplantation of an aortic segment can always be performed in less than an hour, while the circulation can go on for several days through the tube before coagulation occurs.

It is a safe and convenient method for the descending aorta. It can be probably used also on the ascending aorta.

The *lateral* diversion consists in establishing a communication between the left ventricle and the descending aorta, or between two parts of the aorta. I have used only the first method. The anastomosis between the left ventricle and the descending aorta is made by means of a paraffined rubber tube or of a large jugular vein preserved in cold storage. One end of the vessel or of the tube is inserted into the apex of the left ventricle and fastened. The other end is inserted into the descending aorta. The ascending aorta is clamped. Then the blood goes directly from the left ventricle to the descending aorta. The direction of the blood stream is reversed through the upper part of the descending aorta, which acts temporarily as an ascending aorta. There is, therefore, no danger of anæmia of the brain. But the operation is still very dangerous because the details of the method are not sufficiently worked out. The mortality was heavy. Before being safe the technic must be considerably improved. Nevertheless, I succeeded several times in anastomosing the left ventricle to the descending aorta and in reversing the circulation through the upper part of the descending aorta, after clamping the arch of the aorta just above the heart. In one experiment the anastomosing tube was very much narrower than the arch of the aorta. As soon as the ascending aorta was clamped, the heart began to beat slowly, and the blood passed with a strong thrill from the ventricle to the descending aorta. In the brachiocephalic arteries pulsations and a marked thrill were felt. The coronary vessels were dilated. The circulation was thus maintained for eight minutes. The animal remained in excellent condition, and when the clamp and the tube were removed, the circulation was re-established in its normal direction. Nevertheless the lateral diversion is still very much more difficult and dangerous than the aortic intubation.

Although my technic was being developed during the course of the experiments and is still far from being perfected,

some new and permanent results were observed. In one experiment the ascending aorta of a dog was incised and sutured by Jaboulay stitches. Two months and a half after the operation, the vessel was examined. The wound had healed perfectly and the scar was very small. In six experiments the transverse suture of the descending aorta was performed, after complete or incomplete section. One dog died of secondary hemorrhage a few weeks after the operation. The others recovered completely. One of them was chloroformed two months and a half after the operation. The anastomosis was found normal without dilatation or stenosis. The remaining dogs are in normal health more than five months after the operation. The patching of the aorta with a piece of vein was made twice. The animals died eight days and twelve days after the operation of secondary hemorrhage. The accident was due to necrosis of the flap in the one case and in the other to the tearing of the flap by a stitch. These complications can be prevented by slight modifications of the ordinary technic. I performed once only the graft of a complete venous segment between the cut ends of the descending aorta. The operation was performed five months ago. The animal is still in excellent health and there is no modification of the femoral pulse. It is, therefore, probable that with the help of the methods for temporarily diverting the blood, certain aneurisms of the aorta on man can be removed and replaced by a piece of vein.

I attempted also to find out some method for the treatment of valvular diseases and localized sclerosis of the coronarian arteries. Theoretically, many operations can be performed on the heart,—incision and dilatation of stenosed valves, cuneiform resection and stenosis of the upper part of the ventricle in case of mitral insufficiency, curettage of endocardiac vegetations, grafting of new vessels on the auricle and ventricle, collateral circulation between two cavities of the heart, aorto-coronarian anastomosis, etc. The development of these technics is not far advanced for I have studied the conditions under which the operations must be performed rather

than the operations themselves. Plastic operations on the
heart are not very much more difficult than on any other parts
of the body. But to perform the operations without disturbing
in an irreparable manner the functions of the nervous system
and of the heart itself is a very complicated problem.

The cardiac operations can be artificially divided into three
classes: operations which do not require the hæmostasis of
the heart, operations which require the hæmostasis for a very
short time, and operations which require the hæmostasis for a
longer time and the stopping of the heart.

1. Several operations can be performed without the help
of the temporary hæmostasis, such as digital exploratioh of
the ventricles or the auricles, dilatation of the mitral valve,
dissection and preparation of a coronarian vessel for anastomo-
sis, incomplete ventriculectomy and suture, etc. I tried to
develop an operation for mitral insufficiency which could be
performed without opening the heart. It consists of producing
a slight stenosis of the upper part of the left ventricle. It
can be obtained by a partial cuneiform resection cf the wall
of the ventricle just below the coronary artery. A dog which
has undergone this partial ventriculectomy two months ago is
still in good health.

2. In the operations of the second class, the cavities of
the heart are open for about one minute, during which time
it becomes possible to insert and fix a tube or vessel into
the ventricular or auricular cavities, to open largely and to
suture the ventricular wall. It would be feasible also to cut
a mitral or tricuspidian valve, or to perform the curettage of
endocardiac vegetations. I tried to determine what are the best
conditions under which that type of operation must be per-
formed. The hæmostasis can be secured by the clamping
of the venæ cavæ as advocated by Sauerbruch. But it is
simpler to clamp with a large soft-jawed forceps the entire
pedicle of the heart. As the interruption of the circulation
does not last more than one or two minutes, it causes no
cerebral complications. The main danger is the occurrence
of fibrillary contractions, which render almost impossible the

re-establishment of normal pulsations. I performed clamping of the heart eight times, with or without cardiotomy, for from one to five minutes. One dog died of respiratory complication, another one of fibrillary contractions, because the needle went through the dangerous region of Cyon, and the others recovered. In case of mitral stenosis, it would be easy to make an incision of the valve. It would be probably possible also to establish an indirect auriculoventricular anastomosis by a vein implanted on the left auricle and ventricle. I succeeded in fastening temporarily a tube into the left auricle and ventricle. The blood was circulating through it with a strong thrill. At the end of the operation, the tube was removed. The animal remained in good health.

3. To the third class belong the operations requiring the interruption of the circulation for a longer time. They would consist of more complicated plastic operations on the cardiac wall, and of the operations on the coronarian arteries. In certain cases of angina pectoris, when the mouth of the coronary arteries is calcified, it would be useful to establish a complementary circulation for the lower part of the arteries. I attempted to perform an indirect anastomosis between the descending aorta and the left coronary artery. It was, for many reasons, a difficult operation. On account of the continuous motion of the heart, it was not easy to dissect and to suture the artery. In one case I implanted one end of a long carotid artery, preserved in cold storage, on the descending aorta. The other end was passed through the pericardium and anastomosed to the peripheral end of the coronary, near the pulmonary artery. Unfortunately, the operation was too slow. Three minutes after the interruption of the circulation, fibrillary contractions appeared, but the anastomosis took five minutes. By massage of the heart, the dog was kept alive. But he died less than two hours afterwards. It shows that the anastomosis must be done in less than three minutes. Perhaps this can be done by using a lateral implantation with a Payr's canula.

The safest method of performing a comparatively long

operation would be to suddenly place the heart in a condition of anæmia. It is well known that the heart loses its excitability very slowly, and that, by using a proper method, it can be revived after a long period of immobility. We do not know exactly for how long it is safe to keep the heart motionless. But the complete stopping of the circulation is more dangerous for the organism than for the heart. The artificial stopping of the circulation and the opening of the cardiac cavity would place the patient in the condition of a man killed by a bullet through the heart immediately after the last respiration and the last cardiac pulsation. This condition is called general death. But it is merely unmanifested life or latent life. The organs, being deprived of circulating blood, are not able to manifest life any longer. But they are still living and could revive, if they were given back their normal physicochemical conditions. Latent life is a very instable state. Without the circulating blood, the tissues are not able to protect themselves against the microbes and the autolytic ferments, which bring about progressively protoplasmic disintegration, that is, elemental death. Immediately after the artificial stopping of the heart, the organism is placed in a·condition intermediary between general and elemental death. This condition is, from a medicolegal standpoint, death. Nevertheless, as long as elemental death has not taken place, the organism can be resurrected. Therefore, the length of the period during which we can stop the heart safely depends on the rate of the development of elemental death. The brain disintegrates first. It is well known that complete ischæmia of the encephalon produces irreparable lesions. Nevertheless, a short interruption of the circulation is not very dangerous. On a dog, the chest was widely opened, and respiration stopped while the pedicle of the heart was clamped for five minutes. Then the clamp was removed and pulmonary ventilation started again by the Meltzer and Auer method. The animal was easily revived. He recovered completely and never presented any psychological troubles. It is known that, when the interruption of the circulation lasts longer, very marked

cerebral lesions occur. I found also, that in an animal, revived after a long period of cerebral ischæmia, the upper nervous centres had disintegrated. The functions of the heart and of the lungs were almost normal. But the animal was paralyzed and appeared to have lost his intelligence and sensitiveness.

The time during which an animal can be maintained without great danger in a condition of latent life is very short. The technic must, therefore, be developed in such a manner that no cardiac operation should last more than five minutes. It seems possible, although dangerous, to stop the circulation and the respiration during that period and afterwards to replace the animal in a condition of normal life.

CONCLUSIONS.

1. The general technic of intrathoracic operations has been improved by the use of a better asepsis and of the Meltzer and Auer method of pulmonary ventilation.

2. Two methods for the diversion of the blood during the operation on the thoracic aorta have been found,—intubation and lateral diversion.

3. For the first time, it has been possible to perform with permanent success a suture of the ascending aorta, several end-to-end anastomoses of the descending aorta, and the grafting on the descending aorta of a segment of vein preserved in cold storage.

4. It has been attempted also to find and to study new cardiac operations, and the conditions under which they must be performed.

The technic of these operations is far from being completely developed. They must not be used on human beings under their present form. Their purpose was only to study some of the principles on which must be based the future surgery of the thoracic aorta and of the heart.

PRELIMINARY NOTE ON THE ACTION OF EHRLICH'S SUBSTANCE 606 ON SPIRO-CHÆTA PERTENUIS IN ANIMALS†

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The usual treatment of yaws with potassium iodid or mercury is very tedious, but the encouraging results already obtained in the treatment of other diseases due to spirochetes, namely, syphilis, relapsing fever and fowl spirillosis, by a single injection of Ehrlich's substance "606"[1] suggest that his principle of "therapia magna sterilisans" may be applied also to yaws.

As a preliminary to the use of any new drug on man, its trial on animals is, if possible, of course, strongly in-dicated on many grounds. Up to the present, the only animal experimentally infected with yaws has been the monkey, various species of which have been infected by Castellani in Ceylon, Ashburn and Craig in the Philippines and Neisser, Halberstadter and Bauerman in Java. A definite lesion results from inoculation, but apparently the reaction is rather superficial, as is indicated by a late weak complement fixation reaction and a relative scarcity of spirochetes.

Recently, I have succeeded in infecting rabbits in the testicle with the spirochetes of yaws and this lesion is an excellent guide to treatment, as the spirochetes are present in myriads in pure culture, the serum reaction is prompt and strong and the organ can easily be removed

1. Ehrlich: Ztschr. f. ärztl. Fortbild., 1909, vi, 721.

and examined. Rabbits, also, are, of course, easier to obtain and to handle than monkeys.

The spirochetes came from a colored soldier returning from the Philippines who had a well-marked case of yaws with three separate general eruptions.[2]

A monkey was first infected on the eyebrow and transfers were then made of the serum of the monkey's yaw to rabbits' testicles. Three rabbits of the first generation became infected in an average of forty-one days and the infection was then carried on from rabbit to rabbit. The spirochetes are now in the seventh generation in the rabbit and the incubation period has fallen to about twelve days. A detailed account of this work will appear in the *Journal of Experimental Medicine* in a short time.

The lesion seems to differ only in degree from a syphiloma of the rabbit's testicle and consists of an edematous tumor in the testicle or epididymis, varying in size from that of a pea to that of an olive. On puncture with a capillary pipette and examination of a drop of the serum with the dark-field microscope a brilliant picture is obtained. Levaditi stained sections are equally striking. The lesion persists four to six weeks and the Wassermann reaction occurs in the first or second week. The general health of the animals is not noticeably affected.

Ehrlich's "606" is dichlor-dioxy-diamido-arseno-phenol; it is a golden yellow powder put up in vacuum tubes, and is given in the form of a disodium salt freshly prepared before each injection. This substance has been built up with the idea that it shall have a selective action for spirochetes and thus open the way to sterilize the tissues by a single adequate dose. Up to the present, no toxic effects of the drug itself have been observed[3]. A quantity of the material has been recently sent to the Rockefeller Institute by Professor Ehrlich for trial in syphilis.

2. Nichols : Proc. New York Path. Soc., 1910, x, 1.
3. Alt : München. med. Wchnschr., 1910, lvii, 561 ; Iversen : München. med. Wchnschr., 1910, lvii, 777.

The following experiments have been made to determine the action of this substance on animals infected with the spirochetes of yaws.

Rabbit 1.—April 1, inoculated in left testicle with material from Rabbit C (first generation from human case). May 6, nodule palpable, spirochetes present; thirty-six days. May 7, spirochetes present. May 9, nodule size of olive; serum negative; 0.1 c.c. injected intravenously with 0.0045 gm. 606 per kilo. May 10, spirochetes not found. May 12, nodule not palpable. May 21, nodule not palpable. June 3, nodule not palpable. June 16, under ether anesthesia, testicle removed; no lesion; spirochetes not found; serum negative.

Rabbit 15.—March 31, right testicle inoculated from Rabbit 7 (second generation). May 7, nodule palpable, spirochetes present; thirty-seven days. May 9, treated as above, intravenously; serum negative; nodule size of olive. May 10, spirochetes not found. May 12, lesion size of pea. May 18, lesion not palpable. May 28, rabbit dead; testicle shows no lesion.

Rabbit 17.—April 14, left testicle inoculated from Rabbit 6 (second generation). May 7, nodule palpable, spirochetes present; twenty-three days. May 9, treated as above, subcutaneously; serum negative; nodule size of pea. May 10, spirochetes not present. May 12, spirochetes not found. May 18, lesion not palpable. June 16, under ether anesthesia, rabbit castrated; no lesion; serum negative.

Rabbit 21.—April 22, right testicle inoculated from Rabbit 9 (third generation). May 14, nodule palpable, spirochetes present; twenty-two days. May 23, serum positive. May 24, spirochetes present; treated as above, intravenously. May 25, spirochetes not found. May 27, serum positive; lesion not palpable. May 31, serum positive. June 14, lesion not palpable; serum positive. June 17, under ether anesthesia, rabbit castrated; no lesion. June 23, serum negative.

In several animals, the serum reaction has persisted a number of days after the removal of the focus of infection.

In all these animals no spirochetes could be found twenty-four hours after the treatment; the lesion disappeared after two or three days and, except in the last case, no complement fixation reaction occurred.

In animals not treated, the lesion, as noted above, persists several weeks at least and the serum reaction

occurs in one or two weeks. These animals were, therefore, permanently cured by a single small dose. Rabbits tolerate a dose thirty times as great as the one given.

Three monkeys infected on the eyebrow have also been treated in a similar way and the lesions have disappeared after twenty-one days.

It would seem worth while to try this remedy in our tropical possessions where the natives are frequently, and white men are occasionally, infected.

LATENT LIFE OF ARTERIES

By ALEXIS CARREL

LATENT LIFE OF ARTERIES.[1]

BY ALEXIS CARREL.

(*From the Laboratories of the Rockefeller Institute for Medical Research, New York*)

PLATES XL–XLIII.

I. INTRODUCTION.

A tissue is in latent life when its metabolism becomes so slight that it cannot be detected, and also when its metabolism is completely suspended. Latent life means, therefore, two different conditions, unmanifested actual life and potential life. Unmanifested actual life is a normal stage of the evolution of all organisms, when they progress from general death toward elemental death. As the metabolism is still going on, although very slowly, it is a temporary condition. Sooner or later cadaveric lesions develop which bring about the complete disintegration of the protoplasm. Potential life consists of a suspension of all actual vital processes. The metabolism being suppressed, no cadaveric changes would take place in the protoplasma. Tissues in a condition of potential life could be preserved outside of the body for an indefinite period of time.

Since the discovery by Loewenhoek, many observers have studied the conditions and the nature of latent life. Their work was of great biological and metaphysical importance, but it did not seem that their observations would ever be of practical interest. Nevertheless, it happens to-day that the evolution of surgery leads to the use of latent life in human therapeutics. In several kinds of operations on blood-vessels, nerves and even the ureter, the graft of vascular segments can be employed. Human vessels must be used, because autoplastic and homoplastic transplantations are more successful than heteroplastic transplantations. Autoplastic grafting of arteries is not possible, since every large artery is necessary to the organism. Homoplastic grafting only can be used. The graft must be taken from an amputated limb, or from

[1] Received for publication March 31, 1910.

the fresh cadaver of an executed criminal or of a man killed by accident. But such an opportunity will certainly not occur precisely when a graft is needed for an operation. The grafts, therefore, must be kept in storage and be ready for use when necessary. Such a preservation of vessels outside of the body can be obtained by placing them in a condition of latent life.

What form of latent life can be used for preserving tissues of mammals outside of the organism? Although unmanifested actual life is only a slow progress towards elemental disintegration, it would be of practical value. It would permit us to preserve the tissues for some time in such a condition that they could be revived if given back their normal physico-chemical conditions. A tissue, immediately after its extirpation from the body or after death, is still living. Its metabolism is going on slowly, while the microbian and autolytic fermentations begin which sooner or later bring about elemental death. There is a period during which the cadaveric lesions are still so slight that they can be interrupted, if the tissues are placed again in their normal condition. The length of the period intermediate between general death and protoplasmic destruction varies according to the nature of the tissues, the temperature and a great many other factors. It is well known that epidermis or periosteum can be kept for a long time outside of the body and be grafted successfully. Sixty years ago, Paul Bert (1) showed that the transplantation of rats' tails, preserved for several days in a small quantity of confined air, at a temperature not higher than $+ 12°$ C., gave positive results. Lately, Jolly (2), examining a few drops of triton's blood, which had been preserved for fifteen days in cold storage and then put back at normal temperature, saw the red blood corpuscles undergoing indirect division. Ehrlich (3) has demonstrated that pieces of tumor which have been frozen for a long time grow again when transplanted. Preservation of arteries in unmanifested actual life would not be perfect, because it retards only and does not suppress the occurrence of cadaveric lesions. It may be, nevertheless, of practical value in surgery.

The ideal method would be to place the vessels in a condition of potential life. The metabolism being completely stopped, the duration of the period of preservation could be indefinite. Many

physiologists do not admit the possibility of the complete suspension of metabolism. They believe that when Doyère (4) dried a rotifer, heated it at 100° C., and revived it afterward, or when Paul Bert grafted successfully upon a rat a rat's tail which had been dried in vacuum and heated at 100° C., the metabolism of these tissues had not been interrupted. It seems, nevertheless, that under certain conditions metabolism can be suspended. Becquerel (5) has preserved seeds for months in a vacuum, at temperatures as low as — 190° C. and — 253° C., and germination occurred afterwards. It seems reasonable to believe that protoplasm can be placed in such a condition that life does not exist actually, but merely potentially. Gautier thought that protoplasm can remain lifeless until it is given its normal physico-chemical condition, like a clock waiting in absolute immobility for the motion which starts its mechanism. Potential life can probably exist in seeds. But it is improbable, although not impossible, that tissues of mammals can be placed in a condition of potential life without undergoing a fatal disintegration.

I began in 1906 some experiments on the preservation of arteries in cold storage (6). It was found immediately that a dog's carotid artery could be preserved outside of the body at a low temperature for several days and transplanted without suffering degeneration of its muscle fibers. Afterwards, I studied more extensively the different methods which can be used in the preservation of vessels in latent life and their biological and surgical results.

II. TECHNIQUE.

The experiments were performed on dogs. The animals were etherized not only during the operation but also during the shaving and the preparation of the skin and the post-operative examination of the vessels. They were operated on by the methods used on human patients.

Each experiment was composed of four stages: extirpation of arteries, preservation, transplantation and examination of the results.

1. *Extirpation of Arteries.*—Segments of common carotid artery were extirpated from medium-sized dogs during life or a short

time after death by ether or chloroform. The arteries were rapidly exposed, dissected, resected and cut in several pieces about three or four centimeters long. They were then washed in Locke's solution and placed in sterilized glass tubes. The extirpation and the handling of the vessels were made under rigid asepsis.

2. *Preservation of Arteries.*—In a first set of experiments, the arteries were killed in order that the evolution of dead arteries and of arteries in latent life could be compared. In a second set, it was attempted to preserve the arteries by freezing and drying. In a third set the arteries were merely preserved in cold storage.

(*a*) *Preservation of Dead Arteries.*—The arteries were killed by being placed into a sealed tube with a few drops of Locke's solution and heated at 80° C. for ten minutes, or by being immersed in glycerin, or in a 2/100 solution of formalin. Afterwards, the formalined vessel was washed in a diluted solution of ammonia and in Locke's solution.

(*b*) *Preservation of Arteries by Freezing or by Drying.*—The tubes containing the arteries were placed in a refrigerator, the temperature of which was oscillating a little above and below — 3° C. The vessels were kept at this temperature for several days. A few hours before the transplantation, the tubes were removed from the first refrigerator and put into another one about at the freezing point. Two hours after, they were taken to the laboratory at a temperature of about + 29° C.

In the preservation by drying, the arterial segments were sealed in a test tube, the bottom of which was filled with calcium chloride. After a few hours, the arteries shrank and became yellowish and, finally, hard and dried like a strand of catgut. They could be preserved in that condition for an indefinite period of time, at the temperature of the laboratory or in cold storage. In one experiment, the tube was placed for several minutes in boiling water. Two hours before the transplantation, the arteries were removed from their tubes and placed in Locke's solution. Progressively, they were imbibed by the fluid, the lumen became visible, the yellowish color disappeared, and after an hour or an hour and a half, they had regained their normal appearance. From a gross anatomical standpoint, they looked exactly like vessels freshly extirpated from an animal.

(*c*) *Preservation of Arteries in Cold Storage.*—The arteries were placed in glass tubes filled with salt solution, Locke's solution, humid air, vaselin, serum or defibrinated blood. The tubes were sealed and deposited in cold storage. Until March, 1908, the temperature of the refrigerator was often a little above the freezing point, but was also oscillating from — 2° or — 3° to + 12° or + 15°. The apparatus was unreliable. Since March, 1908, I have used an ice box, the temperature of which is constantly between 0° and + 1° C.

3. *Transplantation of the Arteries.*—The arterial segments were removed from the tubes, washed in Locke's solution, and soaked in vaselin. One of the carotid arteries of a dog was dissected and cut, and the preserved segment interposed between and sutured to its ends by the ordinary method. Rigid asepsis was employed in these operations. The degree of asepsis varied according to different changes in the organization of the laboratory. From October, 1906, to October, 1908, the degree of asepsis was generally high. From October, 1908, to January, 1909, it was lower and some non-suppurative infections were observed. After January, 1909, asepsis became excellent.

4. *Examination of the Results.*—The results· were examined clinically and anatomically some time after the operation. After a few weeks or a few months the neck of the dog was reopened, the vessel was dissected and the conditions of the circulation were examined. It is important to observe the transplanted segment while the blood is circulating. Its morphology is often different, whether it is functionating or not. Some of the animals were examined in that manner several times. When the time which had elapsed since the operation was considered sufficient, the specimen was removed and examined from a gross anatomical standpoint. Sections of the transplanted arteries were fixed in Zenker's fluid, embedded in paraffin, sectioned and stained with hematoxylin-eosin, Weigert's elastic tissue stain, and with von Gieson's method, when necessary.

III. EXPERIMENTS.

The experiments are divided in three groups: (*a*) transplantation of dead vessels, (*b*) transplantation of frozen or dried vessels, (*c*) transplantation of vessels preserved in cold storage.

(*a*) *Transplantation of Dead Vessels.*—Three experiments were performed. The arterial segments were killed by formalin, glycerin and heat.

Experiment 1. Artery Killed by Formalin.—Small brown dog, No. 313. November 4, 1908. Transplantation on the right carotid artery of a segment of carotid extirpated on November 2, 1908, put in a 2/100 solution of formalin for two days; washed in a weak solution of ammonia, in Locke's solution, and placed afterwards in vaselin. *Microscopical examination.*—Section 141. Artery normal. November 12. No pulsations. Opening of the neck. Obliteration. Marked reaction of the connective tissue. *Microscopical examination.*—Section 166 (Plate XL, Fig. 1). No intima. Media: elastic fibers stretched, no muscular nuclei, a few debris of nuclei in outer layers of the media. Adventitia very much thickened.

Experiment 2. Artery Killed by Glycerin.—Brindle female dog, No. 307. October 27, 1908. Transplantation on the right carotid artery of a segment of carotid preserved in glycerin, and washed in Locke's solution. *Microscopical examination.*—Section 131. Artery almost normal. Nuclei of the muscle fibers retracted and many of them uniformly stained. November 4, 1908. No pulsations. Segment obliterated. Very marked reaction of the surrounding connective tissue. Segment extirpated. *Microscopical examination.*—Section 143. No intima. Media: elastic fibers almost normal. All the muscular fibers have completely disappeared and are replaced by amorphous substance. Very marked thickening of the adventitia.

Experiment 3. Artery Killed by Heat.—Very young white and black male dog, No. 309. October 28, 1908. Transplantation on the right carotid artery of a segment of carotid, extirpated from a dog on October 26. The segment was placed in a sealed tube, the atmosphere of which was humidified by a few drops of Locke's solution, and then placed in cold storage. On October 27, the tube was placed in hot water for 8 minutes, during which time the temperature went from 85° C. to 70° C. Afterwards it was deposited again in cold storage. The examination made on October 28 showed the artery small and retracted, and the muscle nuclei darkly stained and deformed. November 12, 1908. Dissection of the artery. No pulsations in the transplanted segment. Obliteration. Adhesion to the vagus. Marked reaction of the connective tissue. Resection of the artery. *Microscopical examination.*—Section 161. Artery dilated. No intima; lumen filled with thrombus. Media: elastic fibers stretched, broken and partly destroyed. Muscle fibers have disappeared. However, a few debris of nuclei are seen in the outer layers of the media. Adventitia very much thickened.

(*b*) *Transplantation of Dried and Frozen Arteries.*—Four transplantations of dried arteries and two transplantations of frozen arteries were performed.

Experiment 4. Dried Artery.—Dog No. 315. November 5, 1908. Transplantation on the right carotid artery of a segment of carotid extirpated from a dog on October 26. Immediately after extirpation, the artery was put in a

sealed tube half filled with calcium chloride. After a few hours, the artery became dry and assumed the appearance of a piece of catgut. It was then preserved at the temperature of the laboratory. On November 4, the tube was broken and the artery placed in a tube filled with slightly alkaline Locke's solution. On November 5, the vessel was found with its normal appearance, color and consistency. It was put in normal Locke's solution, in vaselin and grafted into the dog. November 12. Dissection of the carotid. Reaction of the connective tissue around the vessel. Obliteration. *Microscopical examination.*—Section 163. No intima; lumen filled with organized thrombus. Media: elastic frame-work normal. No muscular fibers. Adventitia thickened and infiltrated by leucocytes.

Experiment 5. Dried Artery.—Yellow, male, middle-aged bull, No. 320. November 11, 1908. Transplantation on the left carotid artery of a segment of carotid extirpated from a dog on November 5. The segment has been desiccated in a tube of calcium chloride and placed for one hour in Locke's solution just before the transplantation. It resumed its normal appearance. After transplantation and reëstablishment of circulation the vasa vasorum are immediately injected with blood. Normal color, caliber and consistency. *Histological examination.*—November 25. No pulsation on the left side. November 30. Opening of the neck. Obliteration of the transplanted segment. *Microscopical examination.*—Section 184. Lumen filled with thrombus. Intima present in some places and very much thickened. Media dissected in several places by infiltration of red blood corpuscles. Elastic frame-work normal. In many places, the muscular fibers have completely disappeared. In one point of the media, close to the adventitia, a number of muscular fibers are still normal. Adventitia a little thickened.

Experiment 6. Dried and Heated Artery.—Yellow, male, middle-aged bull, No. 320. November 11, 1908. Transplantation on the right carotid of a segment of carotid artery extirpated on November 5, 1908, desiccated in a tube of calcium chloride, heated for 12 minutes at 100° C., and placed for one hour in Locke's solution, just prior to the transplantation. *Microscopical examination.*—Section 154. Artery apparently normal. But under high power, muscle fiber nuclei appear to be deformed. November 25. Normal pulsations. November 30. Opening of the neck. Normal pulsations. Normal appearance of the vessel. No modification of caliber of the transplanted segment. Extirpation of the upper part of the segment and reëstablishment of the circulation by a segment of jugular vein. *Microscopical examination.*—Section 185. No intima. Media: thinner than normal, elastic fibers normal, no muscular fibers. However, a very few elongated nuclei are seen in the more external layers, close to the adventitia. Adventitia exceedingly thickened, about twice and three times thicker than the media, according to the location.

Experiment 7. Dried Artery.—White, black and yellow bitch, No. 324. November 20, 1908. Transplantation on the right carotid artery of a segment of carotid artery extirpated on November 16, 1908, and dried on calcium chloride. *Microscopical examination.*—Section 177. No intima. Adventitia normal. Media: elastic fibers normal, nuclei of muscular fibers more irregular and uniformly stained. November 23. Normal pulsations. June 8, 1909. Dog died at the farm. *Macroscopical examination.*—Dissection of the carotid. No ad-

hesions to the vagus. The anastomoses are easily seen as very narrow brownish lines. Transplanted segment has same color, caliber and consistency as normal carotid. *Microscopical examination.*—Section 236. Intima as thick as the media in some points, composed of layers of connective tissue cells with elongated nuclei and infiltrated by a great many exceedingly thin elastic fibers, especially in its inner parts. Media: interna elastica normal (Plate XL, Fig. 2), no muscular fibers. However, a few elongated muscles are seen between the outer elastic layers. Adventitia a little thickened.

Experiment 8. Frozen Artery.—White and black male dog, No. 149. July 11, 1907. Transplantation on the carotid artery of a segment of carotid extirpated from a dog on July 8. The segment was put in a refrigerator at the temperature of — 3° C. immediately after the extirpation. It was removed from the refrigerator on July 11 at 6 A.M., and put into a refrigerator at a temperature of + 1° C. At 9:10 A.M. it was brought to the operating room at a temperature of 29° C. July 16. No pulsations. Neck opened and artery dissected. Obliteration of the lower anastomosis. Upper anastomosis normal. Wall of the transplanted segment apparently normal. *Microscopical examination.*—Section 51. Transverse section at the level of the middle part of the transplanted segment. No intima. Adventitia very much thickened. Around small pieces of silk, very large foci of leucocyte infiltration. Media deeply modified. In the internal portion all the muscular fibers have disappeared. Between the laminæ of the elastica, a few leucocytes are seen. In the outer layers of the media, a few deformed or uniformly stained muscle cell nuclei still persist. Longitudinal section at the level of the upper anastomosis. The carotid of the host is almost normal, although its intima is infiltrated by leucocytes. Between the ends of the normal and transplanted carotids, there is a large scar. All the muscle fibers of the transplanted carotid have disappeared. A few leucocytes and debris of muscle fibers are seen between the elastic tissue fibers. Typical non-suppurative infection.

Experiment 9. Frozen Artery.—No. 152. July 16, 1907. Transplantation on the left carotid artery of a segment of carotid preserved since July 11, by the method used in Experiment 8. July 25. Normal pulsations. October 1. No pulsation. October 9. No pulsations. Extirpation of the carotid artery. Complete occlusion of the transplanted segment.

(c) Transplantation of Arteries Preserved in Cold Storage.— The arteries kept in cold storage were preserved in different media. The experiments are divided in several classes, according to the nature of the fluid.

In three experiments the vessels were kept in normal saline solution or in Locke's solution.

Experiment 10. Salt Solution.—White bitch, No. 5. October 31, 1906. Transplantation on the right carotid artery of a segment of carotid extirpated from a dog on October 30. The segment was preserved into a tube of 10/1000 sodium chloride solution, and placed in cold storage. The temperature was irregular, a little above the freezing point. December 4. Dissection of the

artery. Normal pulsations on and above the transplanted segment. Adhesions to the vagus. Resection of the segment. The lumen is slightly enlarged, the wall thickened and the anastomoses normal. *Microscopical examination.*—Section 5. Adventitia thickened. Media: muscular fibers of the inner layers of the media have completely disappeared. Between the elastic laminæ, which are normal, there are a number of mononuclear leucocytes. The middle and outer layers of the media are composed of elastic laminæ and of muscular fibers. In the middle part of the media, the nuclei of the muscular fibers are deformed and surrounded by a few leucocytes. No intima. A thin coat of fibrin is adherent to the interna elastica (Plate XLI, Fig. 3).

Experiment 11. Salt Solution.—Small, male fox terrier, No. 8. November 5, 1906. Transplantation between the central end of the right carotid and the peripheral end of the right external jugular vein of a segment of carotid extirpated from a dog on October 30 and preserved in sodium chloride solution and in cold storage. December 5, 1906. Pulsations and thrill of the external jugular. December 19, 1906. No pulsations, no thrill. Dissection of the vessels. Transplanted segment surrounded by thick connective tissue. Very strong adhesions to the anterior edge of the sterno-mastoideus muscle. Segment occluded by a clot adherent to the upper anastomosis. Lower anastomosis normal. *Microscopical examination.*—Section 168. Adventitia very much thickened. Intima thickened. Media: elastic fibers normal, muscle fibers generally normal. Some of the nuclei are irregular and deformed.

Experiment 12. Locke's Solution.—Black bitch, No. 80. March 6, 1907. Resection of a short segment of the left carotid. Transplantation of a segment of carotid extirpated on February 26, and preserved in Locke's solution. May 3. Dissection of the artery. Normal circulation. No adhesions to the vagus nerve. Appearance of the artery entirely normal. Almost no evidence of the previous operation. May 15, 1908. Dissection of the artery. Slight adhesions to the vagus. Normal circulation. The location of the transplanted segment is hardly recognizable. Resection of the carotid. The location of the transplanted segment becomes evident, because the normal artery contracts itself, while the segment does not. Anastomoses normal, almost invisible. Intima smooth and glistening. *Microscopical examination.*—Section 126. Adventitia normal. Media: the normal elastic frame-work has practically disappeared. It is replaced by a great many small elastic fibers, diffused through the media. No muscular fibers. Intima irregularly thickened, composed of dense connective tissue, covered by endothelium.

In five experiments, the arteries were kept in sealed tubes, the atmosphere of which was humidified by a small quantity of Locke's solution.

Experiment 13. Confined Humid Air.—Yellow, male cur, middle-aged, No. 208. January 14, 1908. Transplantation on the right carotid artery of a segment of carotid artery extirpated from a white bull bitch, No. 204, on January 10, 1908, and kept in a sealed glass tube, the atmosphere of which was humidified by a few drops of Locke's solution. The tube was put into a refrigerator, the temperature of which oscillated between 0° and about 10° C.

February 20, 1908. Dissection of the carotid. Normal pulse. Dog sent to the farm. May 25, 1909. Dog normal. July 22, 1909. Killed in a fight with other dogs. *Microscopical examination.*—Section 244. Upper part of the trans-

planted segment. Intima very much thickened. In one place, it is almost as thick as the media. It consists of two coats of connective tissue cells, the internal coat being composed of transverse fibers, the external of longitudinal fibers. In the inner part of the intima, several layers of elastic fibers have developed. Media: elastic fibers normal, but more closely distributed than normally. A few nuclei of muscle fibers in the outer layers of the media. The largest part of the media is completely deprived of muscle fibers. The inter-elastic spaces are filled with amorphous substance. Adventitia thickened.

Experiment 14. Confined Humid Air.—White and black young male fox terrier, No. 216. January 24, 1908. Transplantation on the right carotid artery of a segment of carotid extirpated on January 10, from Dog No. 204. *Microscopical examination.*—Section 75. Many muscular fibers, nuclei deformed and uniformly stained. November 14, 1909. Dog in excellent condition. Dissection of the right carotid. Slight adhesions to the vagus. Normal pulsations. Caliber of the carotid normal. By a close examination, the transplanted segment can be found. The location of the anastomoses can also be detected. Resection of the carotid. The transplanted segment becomes immediately very apparent because it does not contract itself, while the normal part of the carotid does. Internal surface of the transplanted segment and anastomoses are smooth and glistening. *Microscopical examination.*—Section 257. Intima is composed of dense connective tissue and is very much thickened. In a few places it is thicker than the media. Media is composed of its normal elastic framework and of an amorphous substance. All the muscle fibers have disappeared. Adventitia thickened.

Experiment 15. Confined humid air.—Male bull dog, No. 265. April 1, 1908. Transplantation on the right carotid of a segment of carotid extirpated from a male dog on March 10. Temperature of the refrigerator oscillating between — 2° C. and + 10° C. May 6, 1908. Dissection of the carotid. Artery is obliterated. Section 102. Lumen of the vessel filled with organized thrombus. Adventitia thick and well vascularized. Media has kept its normal thickness. Elastic fibers and interna elastica normal. Nuclei of muscular fibers normal. Many of them have still their vesicular appearance and their regular shape. Some others are irregular.

Experiment 16. Confined Humid Air.—White, female fox terrier, No. 267. April 2, 1908. Transplantation on the left carotid, of a segment of carotid extirpated from a male dog on March 10. April 9. Dissection of the artery. Normal pulse. Normal size and consistency of the transplanted segment. Its caliber is slightly larger than normal. October 15, 1908. Dog chloroformed. Dissection of the carotid arteries. Arteries are absolutely normal. No evidence of the previous operation. Anastomosis cannot be located. After longitudinal opening of the vessel, it is possible to locate the anastomosis and the transplanted segment, which has become absolutely identical with the other parts of the vessels (Plate XLI, Fig. 4). Section 260. *Microscopical examination.*—Longitudinal section at the level of the upper anastomosis. Intima is very much thickened, almost as thick as the media. It is composed of elongated cells, the nuclei of which assume the appearance of muscle cell nuclei. Media is composed of elastic frame-work, amorphous material and cells with elongated nuclei. Adventitia normal.

Experiment 17. Confined Humid Air.—Male dog, No. 268. April 3, 1908. Transplantation on the left carotid of a segment of carotid extirpated from a young male dog on March 16. May 7, 1908. Segment obliterated. *Microscopical examination.*—Section 101. Lumen filled with an organized thrombus. Adventitia thickened and vascularized. Elastic laminæ destroyed in several places. Muscular fibers normal. Infiltration of leucocytes in the media and the adventitia, which is very much thickened.

Experiment 18. Confined humid air.—Long-haired, black, male dog, No. 286. May 28, 1908. Transplantation on the left carotid of a piece of dog's carotid artery, extirpated on March 16, 1908. For several weeks, the temperature of the cold storage was irregular, going below the freezing point from time to time. October 15, 1908. Animal etherized. Carotid artery exposed. No evidence of anastomoses. By a very close dissection of the artery, two very indistinct lines are seen on the surface of the wall. The diameter and the appearance of the transplanted segment are identical with those of the carotid artery. The adaptation of the transplanted segment to the artery is absolutely perfect. The artery is extirpated. As soon as the circulation stops, the limits of the segment become evident, because the artery contracts its wall while the transplanted segment does not. *Microscopical examination.*—Section 122. Intima very much thickened. At one point, it is thicker than the media. No endothelium, except in a few places. Media thinner than normal. Interna elastica normal. Other laminæ are indistinct. Media is composed chiefly of diffuse elastic fibers. No muscular fibers. Adventitia a little thickened (Plate XLII, Fig. 5).

Experiment 19. Confined Humid Air.—White and yellow, male fox terrier, No. 291, October 2, 1908. Graft on the left carotid artery of a segment of dog's carotid artery, extirpated on March 16, 1908.

The segment is a little modified. Its wall is softer and thinner than usual. It looks like a venous wall. Nevertheless, after reëstablishment of the circulation, the vasa vasorum are immediately injected with blood. They are larger than normal without lateral hemorrhages. October 5. Neck of the dog enlarged. Incision. Hematoma. Ablation of the clots. Large hemorrhage. Slight necrosis of the wall near the upper anastomosis. Ligature of the carotid and extirpation of the transplanted segment.

Experiment 20. Confined Humid Air.—Long-haired yellow dog, No. 293. October 3, 1908. Transplantation on the carotid of a segment of dog's carotid extirpated on March 16. October 15. Segment very adherent to the vagus. Distended and obliterated. The wall is dark, but has kept in some places its normal appearance. The wall is necrosed. The vessel is extirpated and the animal recovers.

Experiment 21. Confined Humid Air.—White and yellow, male fox terrier, No. 292. October 15, 1908. Transplantation on the right carotid of a segment of carotid extirpated from a dog on September 29. *Microscopical examination.*—Section 120. Muscle cells nuclei deformed, retracted and uniformly stained. October 19, 1908. Dissection of the carotid. Normal pulsations. No adhesions of the segment to the vagus. Resection of a small part of the transplanted segment. Suture and reëstablishment of the circulation. *Microscopical examination.*—Section 124. No intima. Media: elastic fibers normal. All muscular fibers, without exception, have disappeared. Adventitia: a few

debris of nuclei. No wandering cells. On the external side of the adventitia, connective tissue of the host forms, in some places, a coat infiltrated with leucocytes.

Experiment 22. Confined Humid Air.—Medium-sized, white fox terrier, No. 310, October 29, 1908. Transplantation on the right carotid artery of a segment of carotid extirpated on October 22. *Microscopical examination.*—Section 134. No intima. Media: many muscle fiber nuclei uniformly stained. Adventitia normal. November 12, 1908. Marked reaction of the connective tissue around the transplanted segment. Segment obliterated. *Microscopical examination.*—Section 165. No intima: lumen filled with thrombus. Media: elastic fibers normal. A great many nuclei of the muscular fibers are normal. Many polymorphonuclear leucocytes in the outer coats. Adventitia very much thickened and infiltrated by leucocytes.

Experiment 23. Confined Humid Air.—Young, white and black, male dog, No. 309. October 28, 1909. Transplantation on the right carotid artery of a segment of carotid extirpated on October 26. *Microscopical examination.*—Section 132. Normal artery. November 12. Non-suppurative inflammation of the wound. Marked reaction of the connective tissue around the artery. Adhesion to the vagus. *Microscopical examination.*—Section 162. Intima and media absolutely normal. Adventitia thickened (Plate XLII, Fig. 6).

Experiment 24. Confined Humid Air.—Small, brown, male dog, No. 313. November 4, 1908. Transplantation on the left carotid artery of a segment of carotid preserved since November 2, 1908. *Microscopical examination.*—Section 142. Nuclei of many muscle fibers deformed. November 12. Opening of the neck. Non-suppurative infection. Marked reaction of the connective tissue around the vessel. Segment obliterated. *Microscopical examination.*—Section 167. Thrombus: media infiltrated by leucocytes. Many muscle fibers have disappeared. Many also are still normal. Elastic frame-work normal. Adventitia thickened and infiltrated by leucocytes.

Experiment 25. Confined Humid Air.—Small-sized, yellow dog, No. 302. October 21, 1908. Transplantation of the left carotid of a segment of dog's carotid extirpated on September 29, 1908. *Microscopical examination.*—Section 125. No intima. Media: elastic fibers normal. Nuclei of the muscular fibers retracted. Many of them are uniformly stained and have lost their vascular appearance. November 25, 1908. Dissection of the artery. No adhesions. No reaction of the connective tissue. Normal circulation. Extirpation of the segment, which is harder and whiter than normal. *Microscopical examination.*—Section 180. Adventitia normal. Intima very much thickened and composed of elongated connective tissue cells. Media partly composed of an amorphous substance, highly refractive, which is broken in several places. This substance is disposed in two distinct bands. Between them, there are some normal muscular fibers.

Experiment 26. Confined Humid Air.—Yellow and white, young, male dog, January 5, 1909. Transplantation on the right carotid artery of a segment of carotid extirpated from a dog on February 4, 1909. The color of the segment is yellowish. It flattens more easily than normally. After reëstablishment of the circulation, the vasa vasorum are immediately filled with blood. January 6, 1909. Dog is found dead. *Autopsy:* diffuse broncho-pneumonia.

Experiment 27. Confined Humid Air.—Yellow, male' dog, No. 326. November 25, 1908. Transplantation on the left carotid artery of a segment of carotid artery extirpated from a living young dog on November 9, 1908. January 21, 1909. Opening of the neck. Transplanted segment has same caliber and consistency as normal vessel. Its color is a little whiter. Normal pulse. Extirpation of a piece of transplanted segment and end to end suture of the artery. *Microscopical examination.*—Section 181. Media thinner. Adventitia and intima very much thicker. Adventitia formed of two parts: internal, composed of irregular connective and elastic fibers; external, composed of regularly disposed large connective tissue fibers. Media composed of elastic fibers of normal appearance but more closely distributed on account of the disappearance of almost all the muscular fibers, which are replaced by an amorphous substance. In the outer and inner layers of the media, a few muscular fibers can be detected. Some nuclei are normal, some others have lost their vesicular appearance, or are broken and very irregular in shape. Intima elastica normal. Intima very much thickened and covered with endothelium. Lumen of the vessel about same size as before transplantation. October, 1909. Opening of the neck. Normal circulation. Dog sent back to farm.

In ten experiments, the arteries were preserved in defibrinated blood or serum.

Experiment 28. Defibrinated Blood.—Dog No. 66. February 18, 1907. Resection of a small segment of aorta. Graft between the ends of a segment extirpated on February 15, and preserved in defibrinated blood and in cold storage. Suture by patching of the inferior mesenteric artery on the wall of the transplanted segment. February 23. Normal circulation. Extirpation of the aorta and transplanted segment. Anastomoses normal. Very thin coat of fibrin on a part of the wall of the transplanted segment, and on the line of suture of the upper anastomosis.

Experiment 29. Serum.—Male fox terrier, No. 310. October 29, 1908. Transplantation on the left carotid artery of a segment of dog's carotid extirpated on October 27, 1908 and preserved in dog's serum. November 12, 1908. Marked reaction of the connective tissue. Adhesions to the vagus. No pulsations. Obliteration. *Microscopical examination.*—Section 164. No intima. Media: elastic framework and muscular fibers entirely normal. Adventitia a little thickened and infiltrated with round cells.

Experiment 30. Defibrinated Blood.—Yellow, black and white dog, No. 378. January 26, 1909. Transplantation on the left carotid of a piece of carotid preserved in defibrinated blood since January 22, 1909. Dog is sent to the farm a few days after the operation. Died of pneumonia on February 15, 1909. *Macroscopical examination.*—No reaction of the connective tissue. No adhesions to the vagus. Caliber, color and consistency of the transplanted segment normal. *Microscopical examination.*—Section 216. No intima. Media: elastic framework normal. The muscle fibers of the inner layers have disappeared. The fibers of the middle and outer layers are normal. Adventitia a little thickened.

Experiment 31. Defibrinated blood.—White and black, female fox terrier, No. 385. January 29, 1909. Transplantation on the left carotid of a segment of carotid preserved in defibrinated blood since January 22. November 30, 1909. Dissection of the left carotid. Normal pulsations. Marked adhesions to the

vagus. Extirpation. Transplanted segment similar to the other parts of the carotid. *Microscopical examination.*—Section 265. No intima. Media: abnormally thin and composed of elastic fibers and amorphous substance. All muscle fibers have disappeared. Adventitia very much thickened.

Experiment 32. Defibrinated Blood.—Small, black and tan, male dog, No. 392. February 5, 1909. Transplantation on the left carotid of a segment of carotid preserved in defibrinated blood since January 22, 1909. *Microscopical examination.*—February 5, 1909. Section 212. Some muscle fiber nuclei uniformly stained and deformed. October 18, 1909. Dog died at the farm. *Macroscopical examination.*—Dissection of the artery. Transplanted segment of normal caliber. Slight adhesions to the vagus. Consistency of the wall harder. Its color is modified by the presence of yellow and hard patches. Anastomoses smooth and glistening. The patches are located between the media and the intima. The wall is thickened, but the lumen of the transplanted segment and of the anastomoses is not modified. *Microscopical examination.*—Section 253. Adventitia normal. Media deeply modified. In some parts it is very thin on ac‐count of the complete disappearance of the muscular fibers. It is composed only of the elastic framework and an amorphous substance. In some other parts, there are still some elongated cells between the elastic laminæ. The intima is exceedingly thickened. It is, in certain parts, five or six times thicker than the media and is composed of dense connective tissue. In the middle of the adventitia, the connective tissue has undergone calcareous degeneration.

Experiment 33. Defibrinated Blood.—Black, male dog, No. 402. February 11, 1909. Transplantation on the left carotid of a segment of carotid extirpated February 4, 1909. November 14, 1909. Dog in excellent condition. Dissection of the artery. Normal size, normal pulsations, no adhesions to the vagus. The location of the transplanted segment cannot be determined for the anastomoses are invisible. Resection and longitudinal incision of the artery. The lines of union are easily seen on the internal surface of the vessel. Anastomoses perfect. *Histological examination.*—Section 258. Intima a little thickened. Media: elastic framework normal. All the muscular fibers have disappeared. Adventitia very much thickened.

Experiment 34. Defibrinated Blood.—Brown dog, No. 408. February 17, 1909. Transplantation on the left carotid of a segment of carotid preserved for 48 hours in defibrinated blood and suprarenal. October 10, 1909. Dog killed in fighting other dogs. *Macroscopical examination.*—No evidence of the operation. No reaction of the connective tissue. No modification of caliber, color and consistency of the transplanted segment which cannot be located. Carotid artery is extirpated and longitudinally opened. The location of the segment is detected by a slight difference in the color of the intima which is a little yellowish. *Microscopical examination.*—Section 226. Adventitia normal. Media: elastic framework normal. No muscular fibers. Intima thickened and composed of dense connective tissue.

Experiment 35. Defibrinated Blood.—Male dog, No. 410. February 18, 1909. Transplantation on the left carotid of a segment of carotid preserved in defibrinated blood and thyroid since February 15, 1909. February 27, 1909. Opening of the neck. Circulation normal. Intense reaction of the connective tissue. June 5, 1909. Dog killed by accident. *Macroscopical examination.*—Very

marked adhesions of the transplanted segment to the vagus. Segment normal, although its caliber is slightly larger than that of the carotid. Anastomoses perfect. *Microscopical examination.*—Section 235. No intima. Media: normal elastic framework. No muscular fibers, although very few elongated cells are seen between the elastic laminæ. Adventitia very much thickened and composed of dense connective tissue. In several places the adventitia is thicker than the media and contains a great number of small vessels.

Experiment 36. Defibrinated Blood.—Black and tan bitch, No. 419. March 2, 1909. Transplantation on the left carotid of a segment of carotid extirpated on February 4, 1909. November 14, 1909. Dissection of the left carotid. The location of the transplanted segment can hardly be determined. The carotid has everywhere the same appearance and consistency. Resection of the artery. As soon as the circulation is stopped, the location of the segment becomes apparent, because it contracts less than the normal parts of the artery (Plate XLIII, Fig. 7). *Microscopical examination.*—Section 259. Intima a little thickened and containing a few elongated nuclei, assuming the appearance of muscle cells nuclei. Media: elastic frame-work normal. No muscular cells in the outer layers of the media. Cells with elongated nuclei in the middle and inner layers. Adventitia normal.

Experiment 37. Defibrinated Blood.—Yellow, male dog, No. 448. May 5, 1909. Transplantation on the right carotid of a segment of carotid extirpated on February 4, 1909. November 30, 1909. Dissection of the right carotid. No pulsations. Segment obliterated and transformed in a fibrous tract.

In four experiments, the vessels were preserved in vaselin.

Experiment 38. Vaselin.—Male dog, No. 450. May 6, 1909. Transplantation on the left carotid of a segment of carotid artery preserved in vaselin and in cold storage for 24 hours. August 11, 1909. Dog killed in a fight. *Macroscopical examination.*—Segment is obliterated by a clot of recent formation adherent to the wall at the level of its middle part. Anastomoses normal and free of clot. *Microscopical examination.*—Section 254. Intima slightly thickened. Media: elastic framework normal. Muscle fibers, nuclei normal. However, in a few places, between the two or three inner layers of the elastic fibers, they are missing. Adventitia normal.

Experiment 39. Vaselin.—Light yellow bitch, No. 454. May 12, 1909. Transplantation on the right carotid artery of a segment of carotid extirpated from a dog on May 5, 1909, and preserved in vaselin. November 30, 1909. Dissection of the carotid. No pulsations. Transplanted segment obliterated and atrophied. The ends of the carotid artery are united by a very small vessel. Its wall is very thin and its lumen obliterated by a thrombus.

Experiment 40. Vaselin.—Medium-sized dog, No. 458. May 19, 1909. Transplantation on the right carotid artery of a piece of artery preserved in vaselin since May 5, 1909. October 22, 1909. Dog in excellent health. Incision of the neck. Normal circulations. Carotid normal. Anastomoses almost invisible. Resection of the grafted segment. *Microscopical examination.*—Section 252. Adventitia normal. Media: elastic framework normal. Muscle fibers of the outer layers have disappeared. Muscle fibers of the middle and inner layers normal. Intima thickened, and infiltrated by muscle fibers. (Plate XLIII, Fig. 8).

Experiment 41. Vaselin.—White and black dog, No. 460. May 28, 1909. Transplantation on the right carotid of a segment of carotid extirpated on May 5, 1909, and preserved in vaselin. November 29, 1909. Dissection of the right carotid. Normal appearance, normal pulsations. Transplanted segment same caliber as carotid. Anastomoses are easily seen. Resection of carotid and transplanted segment. Intima smooth and glistening. Anastomoses perfect. *Microscopical examination.*—Section 264. Intima very much thickened and composed of connective tissue and of elongated cells which assume the appearance of muscle cells. Media thinner than normal. Almost all the muscle fibers have disappeared. It is composed of elastic fibers, of an amorphous substance, and of a very small number of muscle cells. Adventitia slightly thickened.

IV. RESULTS.

It must be known, from these experiments, whether an artery can be preserved outside of the body in a condition of latent life, and whether a preserved vessel can play safely the rôle of an artery. The results, therefore, will be examined from both standpoints, biological and surgical.

(a) *Biological Results.*—It is impossible to know directly whether or not a tissue which has been preserved for a few days or a few weeks in cold storage is living. There is no chemical reagent of life. The morphology of an artery gives little indication on its biological condition. A pig's carotid artery, which had been kept for six months in cold storage, had the same histological appearance as a fresh artery. There is no morphological difference between a living tissue and a dead tissue. What difference can be detected between the seed which is sterile and the seed which will produce a large tree? The only method of finding out the state of a tissue which is supposed to be in latent life is to replace it under normal physico-chemical conditions and to observe whether it will again manifest life. If two vascular segments which have been preserved in cold storage and assumed the same histological appearance are transplanted into a normal artery, and if after a few weeks one has retained its normal constitution while the other one has degenerated, it can be concluded that the first one was really in latent life while the second one was dead. However, the persistency of the elastic framework and of the connective tissue coats alone of the transplanted segment would not warrant the assumption that the vessel was still living at the time of the trans-

plantation. In such a case, the arterial wall might be possibly a mere outgrowth of the connective tissue from the host, the transplanted segment acting as a scaffold. But the presence of muscle fibers can demonstrate that the wall of the transplanted vessel is still present and is not merely formed by the tissues of the host.

The anatomical change undergone by dead vessels when grafted to a normal artery are shown by the experiments in which arterial segments killed by heat, formalin and glycerin were used. The dead vessels were examined just before being transplanted. The artery killed by formalin was absolutely normal. In the segments killed by heat and glycerin, the muscle cell nuclei were well stained but more or less deformed and retracted. As a whole, the vascular wall had kept an almost normal appearance. These vessels were transplanted into normal arteries and examined again a short time after the operation. In every case, a complete change had taken place. The segment, killed by formalin, spent eight days on the carotid of a dog. During this time, all the muscle fibers of the media disappeared (Fig. 1) and were replaced by amorphous material, while the elastic framework remained normal. The perivascular connective tissue of the host was very much thickened and was building a new wall around the dead structure. The artery killed by glycerin underwent similar changes. All the muscle fibers had disappeared eight days after the operation. Fourteen days after the transplantation, the media of the artery killed by heating was composed of stretched and broken elastic fibers, and of amorphous material, while the adventitia was thickened. These results are similar to those obtained previously by Levin and Larkin (7) in the transplantation of devitalized arterial segments.

These experiments show that a dead vessel undergoes, when transplanted, a rapid degeneration of all its muscle fibers (Fig. 1), while the elastic framework can remain normal. The change takes place in less than eight days. The host reacts against the dead vessel by forming around it a tube of dense connective tissue. A devitalized vessel acts as a foreign body, which can be progressively resorbed and replaced by the tissues of the host.

The arteries which were preserved by freezing behaved like dead

vessels. In Experiment 8, the transplanted segment was examined five days after the transplantation. All the muscle fibers had already disappeared and were replaced by amorphous material. The second experiment was also unsuccessful. These negative results do not mean that frozen arteries cannot be revived. It is well known, since the work of Ehrlich, that tumors, which have been frozen, can grow again normally. Other causes than freezing and especially non-suppurative infection may be responsible for the failure of my two experiments.

The results given by the arterial segments preserved by desiccation are very much better. Four experiments were performed. After having been in cold storage for a few hours in a sealed tube filled with calcium chloride, the arteries shrank and became brown and hard like a strand of catgut. In Experiment 6, the tube was then heated at 100° C. for ten minutes. Afterwards, the arteries were put in Locke's solution and regained their normal appearance. Their anatomical condition was practically normal. Nevertheless, they behaved differently after having been transplanted. In Experiment 4, all the muscle fibers, without exception, had disappeared seven days after the operation. The anatomical changes undergone by the vessel resembled very much those of vessels killed by formalin or glycerin. In Experiment 7, where the vessel had been dried, and in Experiment 6, where it had been dried and heated at 100° C., the results were different. The media was almost entirely composed of elastic fibers and amorphous material six months after the operation in the first case (Fig. 2), and nineteen days after the operation in the second case. Nevertheless, in the outer layers of the media, a few elongated nuclei, morphologically similar to muscle cell nuclei, could be detected. In Experiment 5, nineteen days after the operation, there were, in the outer layers of the media, a large number of normal muscle cell nuclei. It shows that some muscle cells had not been killed by the desiccation. This method may perhaps be improved and give better results. It is well known that in the drying of rotifers, a very slight difference of technique modifies widely the results. When a rotifer is dried on a glass plate, it cannot be brought back to life. If it is dried in sand, the resurrection is almost constant. The

rapidity of the desiccation plays doubtless an important rôle in the production of irreparable injuries in the architecture of the tissues of the cells. The fact that nineteen days after the transplantation of an artery which has been dried, many muscle fibers are still normal, shows that desiccation does not kill necessarily the tissues of the artery.

The results of the transplantation of vessels preserved in cold storage are divided in several classes, according to the medium in which the tissues were kept.

In three experiments, the arteries were placed in Locke's solution or in a 10/1000 solution of sodium chloride. In Experiment 10 the artery, kept for twenty-four hours in saline solution, was extirpated thirty-five days after the operation. The muscle fibers of the inner layers of the media had disappeared, but elsewhere they were normal (Fig. 3). The artery used in Experiment 11 was kept six days in saline solution. Forty-four days after the transplantation, the muscle fibers were still in excellent condition. In spite of the toxic action of the sodium ions for the tissues, an artery can be preserved for twenty-four hours and six days in physiological saline solution without being killed. In Experiment 12 a segment of artery was kept for nine days in Locke's solution, and then transplanted into a dog. The result was examined fourteen months after the operation. All the muscular fibers had degenerated.

In fifteen experiments the arteries were preserved in confined humid air. After a period varying from two days to eleven months, they were removed from their sealed tubes and transplanted. When they were examined before the transplantation, their appearance was generally found normal. Even after several months, the gross anatomy of the vessels was very little modified. The histological examination showed that they were often normal.

The results of the experiments are divided in four classes. The first class is composed of Experiments, 19, 20 and 26, in which the vessels were preserved from seven to eleven months outside of the body. Necrosis occurred soon after the transplantation. The second class consists of Experiments 14, 16, 18, 21 and 25 in which the muscle fibers degenerated completely, and were replaced by an

amorphous material (Fig. 5), while the elastic framework remained normal. Compensating changes took place in the adventitia and the intima. Therefore, these vessels behaved very much like vessels killed by heat, formalin or glycerin. The period of preservation of the vessels outside of the body had lasted from fourteen to seventy-two days. The examination of the transplanted vessel was performed from six days to six months after the operation. The third class is composed of Experiments 13, 15, 24 and 27. The arteries were partially killed during the period of preservation, which lasted from two days to twenty-one days. The results of the transplantation were examined from eight days to eighteen months after the operation. Some muscle fibers were still normal even after eighteen months. In the fourth class, composed of Experiments 17, 22 and 23, the media was found to be normal. The arteries had been preserved for two days, seven days, and seventeen days outside of the body. The results of the transplantation were examined twenty-one days and fifteen days after the operation. The muscle fibers had remained normal (Fig. 6).

The marked differences observed in the results are due to several different causes. It is probable that the changes of temperature, the slight infection occurring during the period of preservation and after the transplantation caused many of the lesions found in the vessels. Several of the experiments were made when the temperature of the refrigerator was very inconstant and when the asepsis of the laboratory was doubtful. The preservation in confined humid air is not alone responsible for the observed degenerative changes of the arterial wall.

The preservation of arteries in serum or defibrinated blood was used in ten experiments. The period of preservation lasted from two to thirty-one days. The results were examined from fifteen days to nine months after the operation. In Experiment 28 the histological examination was not made. In Experiment 37 the transplanted vessel was resorbed. The results of the eight remaining experiments can be divided in two classes according to the conditions of the muscle fibers. In two experiments, 29 and 30, where the vessels were preserved for two days and four days, the muscle fibers remained normal. In the six other experiments

all the muscular fibers had disappeared. The elastic framework was normal and the muscular fibers were replaced by amorphous material. In the conditions of my experiments, serum or defibrinated blood acted as defective preservative media and their results were inferior to those of confined humid air.

The results of the four experiments of preservation of vessels in vaselin were very much better, although in Experiment 39, the artery became occluded and atrophied. The accident was due probably to a fault in the technique of the anastomoses and not to the method of preservation. In Experiments 38, 40 and 41, the vessels were kept in vaselin for twenty-four hours, fourteen days, and twenty-three days. In Experiment 38, the artery was examined and the specimen removed three months after the operation. A great many muscular fibers were normal. In the intima there were elongated nuclei which resembled muscle cells nuclei. The results of Experiment 40 were examined five months after the operation. A great many normal muscle cells were found in the media and in the intima. The muscle cells of the outer layers of the media had degenerated (Fig. 8). In Experiment 41, almost all the muscle fibers of the media had disappeared, six months after the operation. A few only were seen in the inner layers of the media. But the intima is thickened and contains many muscle fibers. In these experiments the preservation of the muscle fibers depends on the duration of the period of preservation outside of the body. It must also be noticed that the degenerated fibers are located in the outer part of the media, while they are in its inner part in the other methods, and also that they proliferated into the thickened intima.

All preservative media, salt solution, Locke's solution, confined humid air, defibrinated blood and vaselin permit the vessel to live in latent life under certain conditions, but vaselin appears to be the best medium.

(*b*) *Surgical Results.*—The practical value of a vessel preserved in a condition of latent life depends on its ability to act as a canal for the arterial blood. The only complication which interfered occasionally with the function of the transplanted vessel was thrombosis. The method used in the preservation of the vessel

has a marked influence on the occurrence of obliteration. When the arteries were killed by heat, formalin and glycerin, thrombosis occurred always. The results were a little better when the arteries were frozen or dried. In six operations, two positive results only were observed. In the experiments where the arteries were preserved in salt solution, Locke's solution or confined humid air, the results were very much better. If we eliminate three experiments 'in which the vessels were kept for more than six months in cold storage, we find ten positive results for fifteen operations. The vessels preserved in defibrinated blood gave eight positive results in ten experiments, and the vessels preserved in vaselin, three positive results for four experiments.

Negative results were observed after the transplantation of devitalized arteries. In every case, the segment was found after a few days, occluded by a thrombus. Levin and Larkin (7) also observed frequently the same accident. Nevertheless, thrombosis is by no means a necessary consequence of the graft of a dead artery. It is certain that the circulation can take place normally through an inert structure for some time at least. Once I performed the patching of the abdominal aorta of a dog with a piece of rubber, and the circulation went on normally. Guthrie (8) claims to have observed a normal circulation through a formalined vessel. Levin and Larkin found that ten days after an aortic graft, the circulation was still normal. The artery acts as a foreign body around which the organism builds a new wall of connective tissue (Fig. 1). On the intima of the dead vessels, the blood deposits a thin layer of fibrin, and it is possible that the dead structure is progressively resorbed and replaced by the connective tissue of the host. The devitalized artery is a resorbable foreign body. Nevertheless, it must be emphasized that it is a dangerous method. It can yield occasionally some good results. But immediate or secondary thrombosis occurs very much more often than in the case of transplantation of a living structure.

The results of the transplantation of frozen or dried vessels are better. Frozen vessels were transplanted twice and twice thrombosis occurred a short time after the operation. In four experiments of graft of dried vessels, thrombosis took place twice. The

two positive results were found nineteen days and six months after the operation.   In Experiment 6, an artery which had been dried and then heated at 100° C. for twelve minutes was transplanted.   Nineteen days after the operation, the neck was opened and the transplanted segment was found to have the same appearance, caliber and consistency as the carotid.   The circulation was normal.   As the muscular fibers of the media had degenerated, the vessel had adapted its wall to the pressure by increasing its adventitia which was two or three times thicker than the media. In Experiment 7, the result of the transplantation of a dried vessel was examined six months and eighteen days after the operation. The grafted segment assumed the same color, caliber and consistency as the other parts of the carotid.   The morphologic and functional adaptation of the vessel was excellent.   The media was weakened by the almost complete disappearance of the muscle fibers.   But the elastic framework of the media was still normal, while a great many small elastic fibers had developed in the intima, which was very much thickened (Fig. 2).   These experiments show that perfect functional results can be obtained by transplanting dried arteries.   But the method is not safe, since thrombosis occurred twice in four experiments.   It is possibly due to the technique I used.   It would be necessary to repeat the experiments with a better method, and to try to preserve the dried vessels at a very low temperature, as Becquerel did with seeds.   If tissues of mammals could be placed in potential life, it would be the ideal condition for the preservation of vessels outside of the body.

The results of the transplantation of arteries preserved in cold storage were examined from a few days to eighteen months after the operation.   The proportion of positive results was 66 per cent. when the vessels were kept in Locke's solution and confined humid air, the experiments in which they were preserved outside of the body for more than six months not being counted.   The proportion became 75 and 80 per cent. when the arteries were preserved in defibrinated blood or in vaselin.

The only complication which occurred was thrombosis.   There were no aneurysms nor dilatation of the transplanted segment.   In Experiment 19 a hemorrhage due to necrosis of the segment was

observed. The artery had been preserved for seven months in cold storage. The method cannot be expected to prevent the disintegration of a tissue after such a long time. Thrombosis is really the only complication to be feared. It is difficult to know its causes. Certainly, in several cases, thrombosis was not due to the method of preservation but merely to a fault in the technique of the suture, or to non-suppurative infection. It must be noticed that thrombosis occurred mainly during the period where the asepsis of the laboratory was doubtful. The percentage of positive results can certainly be increased. Most of the dogs were medium and small sized animals. By using larger vessels, for instance, vessels like the human humeral artery, better results could be obtained. Nevertheless, the method of preservation has certainly a marked influence. It seems that vaselin or defibrinated blood are the best media.

The function of the transplanted segment remained normal, even in the cases where the wall has undergone marked histological changes. The new artery adapted itself exactly to the vessel on which it was grafted. The union became so perfect that after a few months, it was difficult to find the location of the transplanted segment. In several experiments, the dissection of the carotid artery did not permit the locating of the graft (Figs. 4 and 7). It was necessary to open the vessel longitudinally in order to see the small linear scars or the faint change of color which were the only evidences of the operation. As soon as the vessel was empty of blood, the transplanted segment could be located easily. When the circulation stopped, the normal wall contracted itself, while the transplanted wall generally did not. The new segment appeared then as a slightly dilated portion of the vessel. In Experiment 16 a segment of carotid had been transplanted on a dog, after having been preserved in confined humid aid and in cold storage for twenty-two days. Six months after the operation, both carotid arteries were dissected. No evidence of the operation was seen. Both carotids had the same color, caliber and consistency. The transplanted segment had become absolutely identical to the normal parts of the carotid. Nevertheless, after the opening of the vessels, it became a little larger than the normal parts of the artery

and could be exactly located. The new arterial segment adapts itself, from a morphological standpoint, to the functionating artery.

In two experiments only the arterial wall underwent pathological modifications. Calcification and atheromatous patches, almost identical to the lesions observed in human arteriosclerosis, developed in its wall. But compensatory changes occurred and the caliber of the vessel was not appreciably modified.

The functions and the macroscopical appearance of the transplanted vessels did not depend at all on its histological architecture. For instance, in Experiment 18 (Fig. 5) the artery, five months after the transplantation, did not show any evidence of the operation. In Experiment 40 (Fig. 8) the artery, five months after the operation, was also normal. The only difference was that in Experiment 40 the wall of the transplanted segment contracted itself slightly after the stopping of the circulation, while in Experiment 18 it did not contract at all. Nevertheless, from a histological standpoint, the differences between these vessels were exceedingly marked. In Experiment 40, the wall was almost normal, although the muscle fibers of the outer layers of the media had disappeared. Compensatory change had taken place in the intima which was thickened and infiltrated by muscle fibers. In Experiment 18, the muscle fibers had been destroyed. The wall was composed only of an almost normal elastic framework and of connective tissue. But compensatory changes, such as thickening of the intima and of the adventitia had taken place, and the resistance and consequently the caliber of the vessel was not modified.

In many other cases the same modifications occurred. When the media was weakened by partial or complete degeneration of the muscular fibers, the wall increased its strength by thickening its intima, or its adventitia, or both media and adventitia. When the lesions of the media were more extensive in one point, the compensatory changes of the intima were marked at the same point. The compensatory changes consisted mainly of a sclerosis of the intima or of the adventitia. Very seldom, new elastic fibers developed in the intima. In the arteries preserved in vaselin, the muscle cells invaded the intima. These changes are adaptive changes which enable the weakened wall to resist the blood pres-

sure. It is the experimental demonstration of the theory advocated by Adami (9) in the development of arteriosclerosis. The thickening of the intima or of the adventitia was surely a compensatory change due to the weakening of the media. When the media was normal, no sclerosis of the other coats took place. The degree of sclerosis was proportional to the extent of the lesions of the media.

Not a single instance of arterial dilatation was observed. If the wall is composed of normal tissue, it reacts against the blood pressure by thickening itself. The function redintegrates quickly the weakened vessel. But its morphology is reëstablished only in the measure necessary to the function.

V. CONCLUSION.

When a segment of artery, killed by heat, formalin or glycerin is transplanted, it undergoes a rapid degeneration. Its muscle fibers disappear while the tissue of the host reacts by building a new wall of connective tissue. When the transplanted vessel has been preserved in a condition of latent life, no degeneration of the wall occurs, or the wall undergoes only partial degeneration. The muscle fibers can keep their normal appearance, even for a long time after the operation. It is, therefore, demonstrated that arteries can be preserved outside of the body in a condition of unmanifested actual life.

The best method of preservation consists of placing the vessels, immersed in vaselin, in an ice box, the temperature of which is slightly above the freezing point.

From a surgical standpoint, the transplantation of preserved vessels can be used with some safety. When the arteries were kept in defibrinated blood or vaselin and in cold storage, the proportion of positive results was 75 and 80 per cent., and this can probably be increased.

EXPLANATION OF PLATES.

PLATE XL.

FIG. I. Formalined carotid artery. Eight days after transplantation. Experiment I. Section 166. Hematoxylin-eosin.

FIG. 2. Carotid artery preserved by drying. Six months after transplantation. Experiment 7. Section 236. Weigert's elastic tissue stain.

PLATE XLI.

FIG. 3. Carotid artery preserved for 24 hours in salt solution and in cold storage. Thirty-five days after transplantation. Experiment 10. Section 5. Hematoxylin-eosin.

FIG. 4. Carotid artery preserved for 22 days in confined humid air and in cold storage. Six months after transplantation. Experiment 16.

PLATE XLII.

FIG. 5. Carotid artery preserved for 72 days· in confined humid air and in cold storage. Five months after transplantation. Experiment 18. Section 122. Hematoxylin-eosin.

FIG. 6. Carotid artery preserved for 2 days in confined humid air and in cold storage. Fifteen days after transplantation. Experiment 23. Section 162. Hematoxylin-eosin.

PLATE XLIII.

FIG. 7. Carotid artery preserved for 26 days in defibrinated blood and cold storage. Eight months after transplantation. Experiment 36.

FIG. 8. Carotid artery preserved for 14 days in vaselin and in cold storage. Five months after transplantation. Experiment 40. Section 252. Hematoxylin-eosin.

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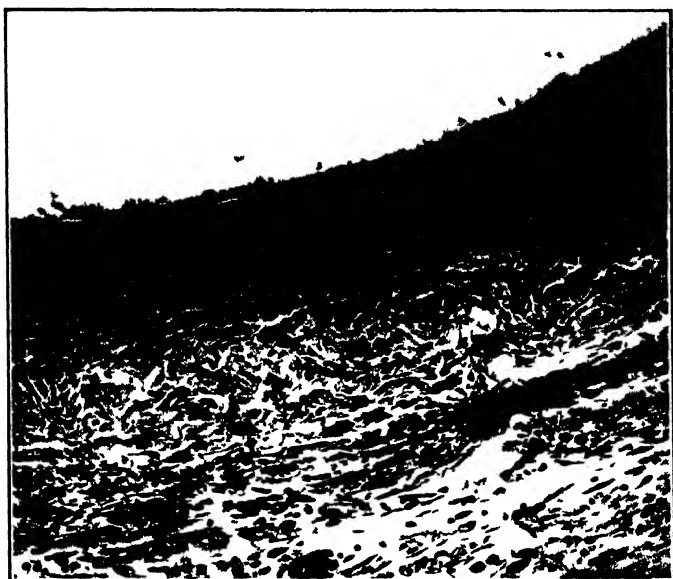
FIG 1
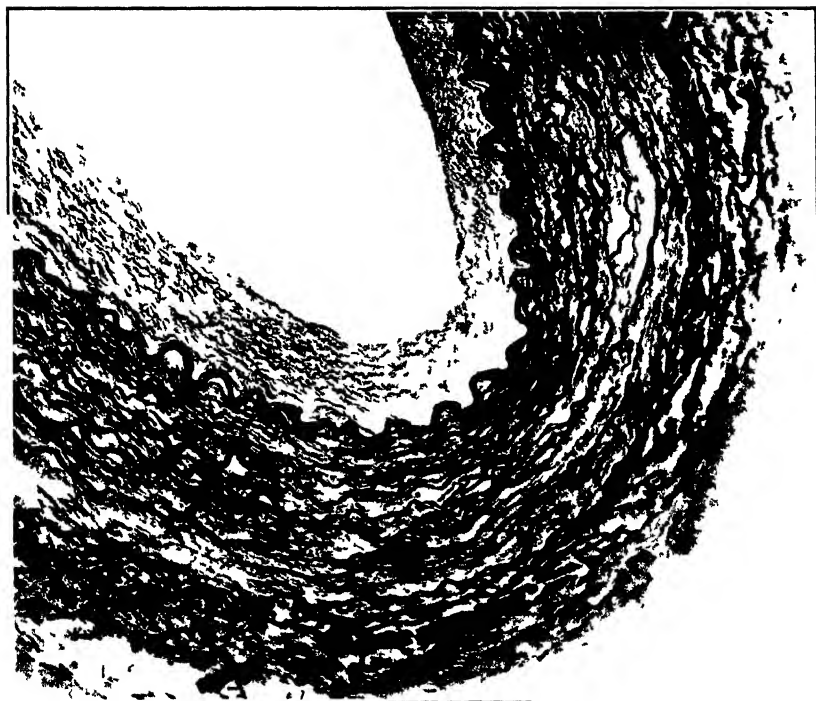
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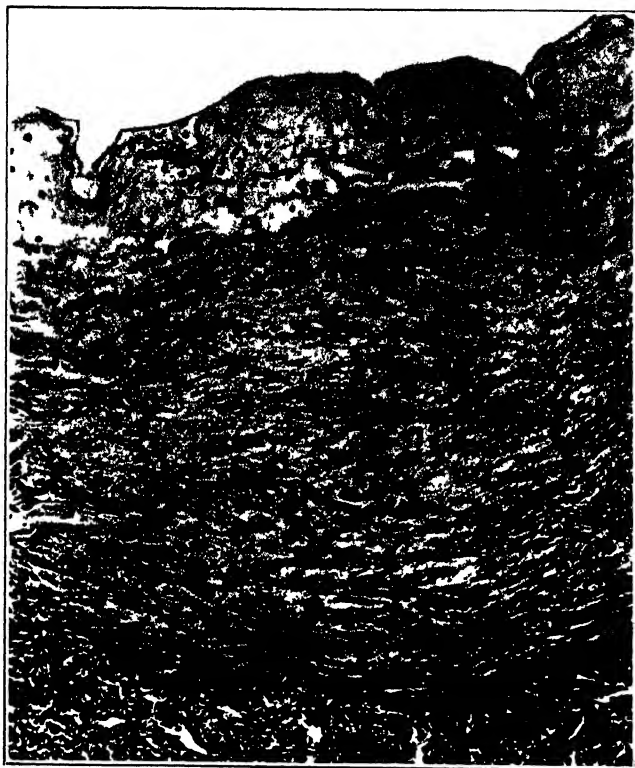
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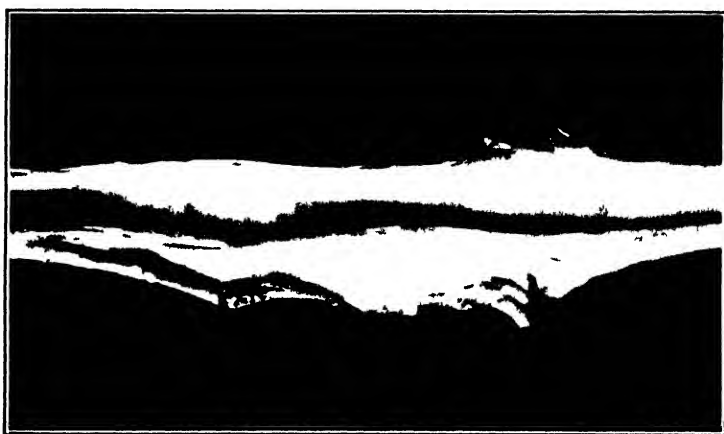
FIG. 4
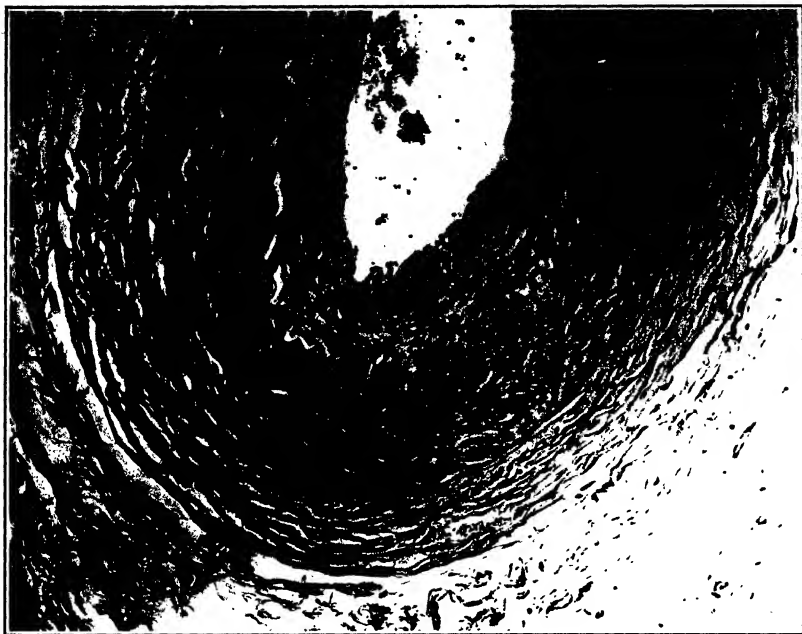
FIG. 5.

FIG. 6.
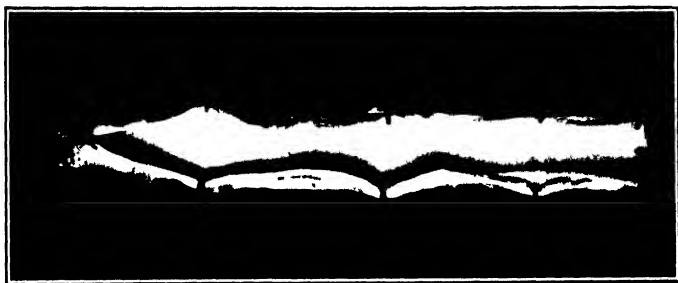
FIG. 7.

FIG. 8.

A STUDY OF AN EXTREMELY PURE PREPARATION OF RICIN.[1]

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In September, 1905, Osborne, Mendel and Harris (1) published a paper entitled, "A Study of the Proteins of the Castor Bean, with Special Reference to the Isolation of Ricin." In their study they found that the toxic substance known as ricin was associated with the coagulable albumin of the castor bean, and that of this protein 0.0005 milligrams per kilo of body weight injected into a rabbit caused death on the seventh day, and a dose of 0.0032 milligrams per kilo injected into a guinea pig caused death in seven days. With their preparation (No. 15), the susceptibility of the rabbit and the cat were about equal, 0.1 milligrams per kilo causing death in two to three days in these two animals. In the guinea pig and dog, 0.5 milligrams per kilo caused death within forty-eight hours. They did not test in any more detail this preparation, which was about one hundred times more toxic than that of Cushny, who had up to this time prepared the most potent ricin. I shall not go into a further discussion of the historical aspect of this subject, but shall proceed to detail my results.

Preparation.—In the preparation of the ricin used in this work I followed exactly the method detailed by Osborne, Mendel and Harris. Although I followed their method in every step, I did not get the proportionate yield of ricin that they obtained, probably owing to their greater experience in the isolation of proteids. Roughly stated, the method consists of extracting the ground beans for forty-eight hours with 10 per cent. sodium chloride solution, two liters of solution to the kilo of meal.[2] This is filtered and

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[2] All the oil has previously been removed from the ground beans by extraction with ether and pressure and then a further grinding.

then dialyzed for four or five days. It is again filtered and the precipitated globulin removed. To the filtrate is then added enough ammonium sulphate to bring it to a 45 per cent. concentration. After standing the precipitate is filtered off and dissolved in water, after which is added an equal quantity of the saturated ammonium sulphate solution. It is then allowed to stand over night and again the precipitate is removed by filtration.[3] The dried precipitate then contains less ammonium sulphate than it otherwise would. After this, the partially dried precipitate is dissolved in water, and enough saturated ammonium sulphate solution is added to bring it to a 33 per cent. concentration. When the precipitate that has been formed by this concentration of ammonium sulphate has settled, it is collected on a filter and then dissolved in water and dialyzed, first for seven days against running tap water and then against distilled water for another week. It is then carefully filtered and the filtrate evaporated as rapidly as possible to dryness *in vacuo.* The coagulable albumin so isolated is the most toxic fraction to be obtained from the proteins of the castor bean. Another fraction of coagulable albumin may be obtained by bringing the filtrate of the 33 per cent. ammonium sulphate concentration to a 50 per cent. concentration and this is also toxic but less so than the precipitate obtained from the 33 per cent. concentration, as the precipitate from the 50 per cent. concentration contains a greater quantity of proteose. All of the results detailed in this paper were obtained with the albumin precipitated by a 33 per cent. concentration of ammonium sulphate. Osborne, Mendel and Harris found in their preparation of this fraction coagulable albumin, 70.64, and proteose, 29.36.

To determine the amount of coagulable protein and proteose in the preparation used in this work, 0.827 gram, calculated as ash free,[4] were taken and dissolved in 10 per cent. sodium chloride solution. It was heated on a water bath for one and a half hours at 94° to 96° C. The coagulum was filtered off, washed, dried and weighed. It contained 0.524 gram, leaving .203 gram of proteose which

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[3] These protein precipitates are best deprived of the excess fluid and salts by placing them between large quantities of filter paper and applying a gradually increasing pressure.

[4] Ash = 5.16 per cent.

gave the following result of percentages: coagulable albumin, 75.46; proteose, 24.54. It will be seen that this agrees fairly well with the figures obtained by Osborne, Mendel and Harris, except that the coagulable proteid is slightly higher.

The Toxicity of the Preparation.—Osborne, Mendel and Harris found that the minimal lethal dose of their most toxic preparation for various animals was as follows: for rabbits, 0.0005 milligram per kilo; for guinea pigs, 0.0032 milligram per kilo; for dogs, 0.5 milligram per kilo (death in forty-eight hours); for cats, .1 milligram per kilo.

The minimal lethal dose of the preparation under discussion was as follows: for rabbits, 0.0001 milligram per kilo; for guinea pigs, 0.0008 milligram per kilo; for dogs, 0.0006 milligram per kilo, for cats, 0.0002 milligram per kilo; for goats, 0.003 milligram per kilo (death in three days).

In the case of the goat this dose caused death on the third day. The autopsy findings in these various animals were similar. These doses are based on intramuscular inoculation. Intravenous injection gave a shorter incubation period, but no great difference in the minimal lethal dose. The time of death could be gauged within rather narrow limits by varying the size of the dose. In rabbits, 10 milligrams per kilo caused death in five to six hours. One hundred milligrams gave exactly the same result, showing that the minimal incubation period is between five and six hours. The pathological lesions were never as marked in these cases of short incubation periods as in those animals dying from the third to the sixth day. Death could be regularly produced up to the sixth day, but after this period, individual variations in the susceptibility of the animals became an extremely disturbing factor. Lesions caused by these extremely potent preparations are identical with those produced by the ordinary commercial preparations.

In 180 animals injected with fatal doses of ricin, the results were as follows:

The autopsy findings were intense hemorrhagic edema at the site of inoculation. The peritoneal cavity always contained a quantity of bloody fluid, varying in amount from 50 cubic centimeters to as much as 200 cubic centimeters. Nine per cent. of the ani-

mals showed the presence of a bloody fluid in one or both pleurae. Three per cent. showed the presence of this fluid in the pericardium. The heart and lungs were normal. About 5 per cent. of the animals showed a localized hemorrhagic area in the thymus; 2 per cent. in the thyroid gland; 15 per cent. in the pancreas; 45 per cent. in the adrenals. The pyloric portion of the stomach and the small intestine showed these lesions in every case. In the small intestines the lesions were more numerous in the duodenum. The lymph nodes of the peritoneal cavity were always enlarged and softened. The liver, as a rule, showed marked congestion and many areas of focal necrosis. The spleen was large, soft, and extremely engorged with blood. The microscopical examination of specimens from these various tissues showed exactly the same picture that Flexner (2) has described. The post-mortem picture is in some particulars similar to that caused by diphtheria toxin, especially the lesions at the site of injection in the adrenals and stomach.

The Hemagglutinin.—This function of the preparation was tested against the red blood cells of horses, rabbits, guinea pigs and dogs. One cubic centimeter of a 1 per cent. suspension of well-washed red blood cells, plus the ricin in one cubic centimeter of .9 per cent. sodium chloride solution. The amount of ricin required to bring about complete agglutination of the above quantity of red blood cells varied between 0.002 and 0.005 for all four species of red blood cells, showing that there was not any greater variation between the minimal agglutinal doses than between the minimal lethal doses.

Keeping Qualities.—At the end of two years and a half this preparation was tested for its toxicity in rabbits, dogs, and guinea pigs, and it was found to be absolutely inert, one-half gram producing no effect in a rabbit. On the other hand, the minimal agglutinating dose was only slightly raised. It now required between 0.005 to 0.009 gram to bring about complete agglutination of one cubic centimeter of a 1 per cent. red blood cell suspension.

Effects of Electric Current.—One gram of this protein preparation was dissolved in twenty-five cubic centimeters of distilled water and placed in a cell, similar to the one described by Field

and Teague (3), with agar-filled tubes as electrodes. A direct current was passed through this solution for five hours, the strength of the current being one hundred and ten volts, and from three to eight milliamperes. After a very short time a white flocculent precipitate appeared about the anode agar. At the end of this time, as no more precipitate was seen to be forming, this precipitate was collected on filter paper and washed thoroughly in distilled water, in which it was insoluble. It was then treated with water containing a slight trace of sodium carbonate, in which it was readily soluble. This preparation was far more active as a hemagglutinin than the original preparation. On the other hand, the minimal lethal dose was markedly decreased. The figures were as follows: .0001 milligram agglutinating dose, as against 0.002 to .005. The minimal lethal dose for rabbits was .002 milligram, as against 0.0001. The filtrate showed a very marked loss in agglutinating value per milligram of protein, but it also showed a loss in the minimal lethal dose as well.

CONCLUSIONS.

1. By following the method of Osborne, Mendel and Harris, we can obtain an extremely potent toxin from the castor bean.

2. It would appear, as a result of testing this preparation at the end of two and a half years, that the agglutinating function and the toxic function are two distinct properties.

3. This result is also borne out by the behavior under the electric current; either we are dealing with two different substances or else with a single substance with two distinct toxiphore groups, one of which is stable and the other labile.

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A Study of Experimental Conditions of Low Blood-Pressure of Non-Traumatic Origin

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A STUDY OF EXPERIMENTAL CONDITIONS OF LOW BLOOD-PRESSURE OF NON-TRAUMATIC ORIGIN*

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The study of experimental conditions of low blood-pressure has been limited for the most part to conditions of shock due to traumatism, thermic influences, or exposure, and aside from pharmacological investigations of the mode of action of certain substances such as arsenic, but few attempts to study, experimentally, low blood-pressure due to toxic agents have been made. The study of the latter should, therefore, be of. interest not only to the internist, in that it may offer an explanation of conditions of collapse or shock occurring in medical as controlled with surgical practice, but also of general importance, in that it supplements the experimental work of Crile[1] and others on surgical shock. Such a study was suggested by the observation of Biedl and Kraus,[2] which we[3] have confirmed, that anaphylactic shock in the dog is characterized by a low blood-pressure resembling in many ways that of surgical shock. This condition, unaccompanied by mechanical injury or hemorrhage, but due to the action of horse-serum on an animal in every way normal except that it had been sensitized to such serum, appeared to us to be analogous to those conditions of suddenly developing low pressure occurring in medical, as contrasted with surgical practice, which are usually associated with a general intoxication. The close analogy between the blood-pressure changes of anaphylactic "shock" and "peptone" intoxication led us to utilize the latter also in our studies.

The main object has been to study the treatment of low blood-pressure, but in order to interpret properly the effects of treatment, it was necessary first to determine the mechanism by which the low pressure was brought about and the effect of the alteration in pressure on the general

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distribution of the blood. That portion of our study which deals with the mechanism of anaphylactic shock has been presented elsewhere.[4] The details of the mechanism of peptone intoxication only will be presented here, but as the mechanism of anaphylactic shock is analogous to that of the peptone effect, the descriptions of the latter illustrate also the former.

DETAILS OF THE STUDY

The change in the distribution of the blood was first studied. All animals were under full ether anesthesia. The pressure was taken from the left femoral artery with a mercury manometer; the peptone solution was injected at body temperature, and slowly, into the right femoral vein. The changes in blood distribution have been determined by oncometric studies of the spleen, kidney and intestine, by a cannula introduced into the right common iliac vein and projecting into the inferior vena cava, by plethysmographic study of a fore limb and by a metal cylinder penetrating the cranial cavity. Each of these pieces of apparatus was connected by rubber tubing with bellows recorders allowing simultaneous records on a revolving drum. It was thus possible in a single experiment to obtain simultaneously, as was frequently done, records of the circulatory variations in the femoral artery, the iliac vein, the kidney, the spleen and the intestine, with also a record of the respiration, or any combination of these, and a record of variations in the volume of a limb, or in intracranial pressure. The temperature of the animals was maintained by a hot-water coil encircling the greater part of the trunk. A record of temperature was obtained by a thermometer in the rectum. Peptone (Witte's) was injected intravenously in the strength of 10 per cent. in 0.85 per cent. salt solution. The solution was allowed to run into the vein slowly from a burette until a sharp fall in pressure occurred. The amount necessary varied considerably, but it usually required 0.2 to 0.4 gm. per kilo to cause a fall in pressure lasting five minutes or more without upward tendency. This phase of our investigation includes ten observations.

OBSERVATIONS FROM THE EXPERIMENTS

It was found that "peptone" intoxication and anaphylactic "shock" are characterized by changes in the circulation which are closely analogous, except that in anaphylaxis the onset is more abrupt and the resulting condition more prolonged. In both a fall in blood-pressure, equal to

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50 to 70 mm. Hg, occurs immediately after injection. This is indèpen-dent of initial change in heart action, though during the continuance of the effect the pulse-pressure is low, the result, apparently, of the small volume of blood passing through the heart. Respiratory distur-bances, except in so far as they are secondary to the medullary anemia consequent on the low general pressure, are absent.

Oncometric studies show a decrease in the volume of kidney, intestine and spleen simultaneous with the fall in arterial pressure. Of these three organs the decrease is as a rule most marked in the kidney and least in the intestine. A very slight initial decrease in brain-volume has been found as well as a slight diminution in the volume of an extremity. This decreased peripheral circulation is accompanied by an accumulation of blood in the large veins of the abdomen. This has been determined by inspection of the liver and large veins, and by the observation that a can-nula introduced into the inferior vena cava and connected with a water manometer shows a moderate increase of pressure equal to 6 to 10 mm. water at the time of fall in arterial pressure, with an almost immediate return to normal level as the blood becomes evenly distributed throughout the large venous trunks.

It is evident, therefore, that the important feature of the condition of low pressure here described is a loss of tone of the vessels of the splanchnic area, resulting in extreme congestion of the large venous trunks. From this condition the animal does not quickly recover, and, as will be shown later, it is not readily influenced by circulatory stimulants. It is essentially the condition frequently characterized as a "bleeding into the veins of the abdomen" and in many respects is analogous to the circulatory disturbance of surgical shock.

Our results with peptone differ somewhat from those of Thompson, in that he found by oncometric studies a secondary rise in intestine-volume. He lays much stress on the influence of gravity, the secondary rise in intestine-volume being most in evidence when the animal is placed on its side and the level of the oncometer lowered. This apparent mechanical error we have avoided by placing the intestinal and other oncometers in as nearly as possible the normal position of the organ under observation. The uniformity of our results leads us to believe that they are not affected by mechanical influences. This view is strengthened by the fact that the organ-volume closely followed the general blood-pressure, and transient changes in the latter brought about in various ways were always accompanied by changes in organ-volume. Also it may be stated that by direct inspection during the stage of low pressure the intestine showed no evidence of congestion except in the large veins; the pallor of the tissue, under such circum-stances, was striking.

5. Thompson, W. H.: Contributions to the Physiological Effects of Peptone when Injected into the Circulation, Jour. Physiol., 1896, xx, 455; 1899, xxiv, 374, 396; 1899, xxv, 1; 1900, xxv, 179.

MECHANISM OF THE PEPTONE REACTION

The mechanism of the "peptone" reaction has been studied extensively. At present we are not especially concerned with the question of the nature of the substance in preparations of peptone which causes the effect on blood-pressure.[6] It may be sufficient to state that the older view, based largely on the experiments of Pick and Spiro,[7] that the physiological effects were due not to the contained proteoses, but to impurities, has been rendered untenable by Underhill's results with purified products; also that the contradictory results depend largely on the animal used; thus the dog is found most susceptible, the cat less so, and the rabbit extremely resistant.

MECHANISM OF CIRCULATORY CHANGES

Concerning the physiological mechanism of the circulatory changes, we have no definite experiments previous to those of Thompson, though Pollitzer had described the blood-pressure changes as due to a vasomotor paralysis affecting chiefly, if not wholly, the splanchnic region, and Grosjean, also, had ascribed to the vasodilatation the chief rôle, but believed it to be the result of a central disturbance. As the result of an extended series of experiments, Thompson concludes that the action of peptone is a direct or peripheral effect on the blood-vessels independent of the vasomotor center. This effect, he thinks, is in all probability a depression of irritability limited to the nervous segment of the neuro-muscular apparatus. His experiments include (1) division of the spinal cord and vagi; (2) excitation of the peripheral end of the cord; and (3) excitation of the divided splanchnic, the cord also being divided. Under all these conditions "peptone" caused a fall in pressure. Also it was observed in the splanchnic excitation experiments that at the period of lowest blood-pressure splanchnic stimulation gave, for a short period of time, no response.

COMPARISON OF PEPTONE AND ANAPHYLACTIC REACTIONS

Our study by physiological methods of the mechanism of changes in pressure due to "peptone," and that associated with anaphylactic shock, includes experiments in which the spinal cord, vagi and cervical sympathetic and splanchnic nerves have been divided. The fall in pressure after peptone injection, as shown in nine experiments, is not influenced by any or all of these procedures; nor do such operative procedures have any effect in preventing the low pressure of anaphylaxis.

In order to eliminate completely any possible central factors in the production of peptone intoxication and anaphylactic shock, decapitated animals were used in addition to those having the cord, vagi, and cervical sympathetic nerves severed. Decapitation may be performed with a comparatively negligible amount of hemorrhage by clamping the common carotid arteries and the internal and external jugular veins simultane-

6. A summary of the literature to 1903 may be found in the excellent account by F. P. Underhill: New Experiments on the Physiologic Action of the Proteoses, Am. Jour. Physiol., 1903, ix, 345.

7. Pick, E. P., and Spiro, K.: Ueber gerinnungshemmende Agenten im Organismus höherer Wirbelthiere, Ztschr. f. physiol. Chem., 1900-01, xxxi, 237.

ously low in the neck, having first ligated the vertebral arteries at the point where they enter their canals in the transverse processes of the cervical vertebræ. After decapitation it was found that the blood-pressure fell to a point averaging one-half of its original level and was maintained there for a period covering the usual length of the experiment, if care was exercised to keep the artificial respiration uniform and to maintain the body heat. With the loss of vagus influence the heart-rate is quickened, the pulse-pressure lowered, and the respiratory waves become well marked. Under such circumstances, however, both peptone and the toxic dose of horse-serum cause a fall of blood-pressure to the level occurring from similar doses in intact animals. The onset of the fall is less abrupt, the low level is reached more slowly and the tendency to spontaneous recovery is markedly decreased.

When, in addition to decapitation, the cord is destroyed, there is a further fall in blood-pressure to a point so low that the heart suffers from lack of blood and death rapidly ensues from failure of the circulation. If, however, this extreme fall and low blood-pressure level is combated, the cardiac anemia prevented, and a circulation sufficient for the observation of changes in pressure is established by increasing the blood-volume by transfusion, it is found that the peptone and anaphylactic reactions may still be obtained. As a result of the extreme peripheral relaxation thus produced, a condition is present in which the vessels are practically a system of non-contractile tubes, cardiac anemia supervenes and death quickly ensues. This demonstrates conclusively that the peptone and anaphylactic reactions may be produced independently of the medullary and spinal centers, by an influence on a vasomotor mechanism located either in the ganglia of the splanchnic area or at the neuromuscular junction in the vessel-walls. The experiments in which the splanchnic nerves and ganglia were destroyed point to the neuromuscular junction as the seat of this action.

While these experiments on animals with the spinal cord destroyed after decapitation have shown that the usual effect on the blood-pressure may be produced without central action, such experiments do not exclude the possibility that the central vasomotor mechanism may play some part in the symptom-complex presented by intact animals. With the object of ascertaining the nature and extent of a possible central action without the complication of the known peripheral effects, we were obliged to devise a means by which the solutions used might be introduced into the cerebral circulation, but not reach the circulation of the body. A prelimi-

nary operation is performed, the details of which are described. elsewhere,[8] whereby vascular communication between the heart and the head and neck is obliterated and the circulation in the head and neck maintained independently by transfusion from a normal animal. Following a short period of irregularity due to the temporary changes in the cerebral circulation incidental to the technic of isolation, the general blood-pressure of the recipient is maintained at a normal level and solutions may be introduced into the cerebral or into the peripheral circulations exclusively without any of the injected material reaching the other circulation. Under such circumstances the venous outflow from the cerebral circulation took place through an incision in the left external jugular vein so that following the injections the brain received normal blood only.

When peptone is injected into the cerebral circulation alone through the carotid anastomosis a lowering of the general blood-pressure occurs, but the depression is less marked and of shorter duration than when the body alone or the entire circulation is involved. Thus, to quote one experiment, the fall in pressure after intracerebral injection was only 30 mm. Hg, whereas, after an injection into the femoral vein, two minutes later, it was 72 mm., the level usually obtained in the intact animal.

When the injection is made into the circulation of the trunk alone or after recovery from the effects of the cerebral injection, it is found that the fall in pressure is more abrupt in its onset than that due to intracerebral injection, and also that the low level is maintained for a longer time. The recovery, however, occurs more quickly than in the intact animal and is apparently due to the strong constrictor impulses coming from the active medullary centers.

Similar results were obtained when horse-scrum was injected into the cerebral circulation and then into the circulation of the trunk of a sensitized dog. After recovery from the slight temporary depression following the cerebral injection, the blood-pressure shows the typical profound depression of anaphylactic shock when the horse-serum is injected into the saphenous vein. This is added evidence that the peripheral mechanism plays the essential part in the condition of anaphylactic shock.

We have therefore felt justified in concluding that in both peptone intoxication and anaphylaxis the action is wholly, or in largest degree, peripheral, either on the nerve endings or on the musculature of the vessels. A number of pharmacological experiments have been made to localize the action still further. These are based on the experiments of

8. Eisenbrey, A. B.: A Method of Isolating the Cerebromedullary Circulation, Proc. Soc. Exper. Biol. and Med., 1910, vii, 113.

Dixon,[9] and Brodie[10] and Dixon with apocodein. This substance in very large doses paralyzes vasomotor nerve endings; and "when its action is complete, pilocarpin, physostigmin and adrenalin have no effect on the blood-vessels or blood-pressure, while barium and digitalis will still constrict the blood-vessels and raise blood-pressure. The first three drugs, it is therefore assumed, act on nerve-endings and the latter two directly on muscle."

As the low pressure of anaphylaxis both in its degree and in permanence resembles closely that due to apocodein, and as Thompson ascribes the peptone action to an effect on the nerve-endings, we have tested the effect of adrenalin and barium in these two conditions. The results are not conclusive. While barium causes an increase in pressure, adrenalin does likewise; in the latter, however, the rise is but slight and never equal, even if percentage values are considered, to the normal adrenalin reaction.[11] Assuming, therefore, that adrenalin acts through the nerve endings, it is evident that in peptone intoxication and anaphylactic shock these structures are not completely paralyzed. Their activity, however, is greatly diminished, not only as shown by the slight adrenalin action, but also by the fact that splanchnic stimulation causes a smaller rise in pressure than in the normal animal. The muscle, on the other hand, is still very active. This is shown not only by the barium effect but also by the effect of nitroglycerin, which, according to Dixon and Brodie, acts only on the muscle and in the two conditions here described causes a further fall in pressure. Furthermore, if a sensitized dog under the influence of apocodein receives an injection of serum, the typical reactions of anaphylactic shock are not obtained. In fact, the blood-pressure is raised a few millimeters by the mechanical effect of the injection and remains so for a period of about one minute, gradually returning to a point slightly below the original level after two minutes. The typical reactions to barium chlorid and nitroglycerin are obtained, but dog's urine which, as we have shown elsewhere,[12] acts on the nerve-endings,

9. Dixon, W. E.: The Paralysis of Nerve Cells and Nerve Endings with Special Reference to the Alkaloid Apocodein, Jour. Physiol., 1903, xxx, 97.

10. Brodie, T. S., and Dixon, W. E.: Contributions to the Physiology of the Lungs; Part II, on the Innervation of the Pulmonary Blood-Vessels; and Some Observations on the Action of Suprarenal Extract, Jour. Physiol., 1904, xx, 476.

11. If adrenalin and peptone are given together the rise due to the former occurs immediately, but is cut short by the peptone effect which continues as usual. This has previously been shown by Hamburger. (Hamburger, W.: The Action of Intravenous Injection of Glandular Extracts and Other Substances on the Blood-Pressure, Am. Jour. Physiol., 1904, xi, 282.)

12. See Pearce, R. M. and Eisenbrey, A. B.: The Mechanism of the Depressor Action of Dog's Urine, with Some Observations on the Antagonistic Action of Adrenalin, Am. Jour. Physiol., 1910, xxvi, 26.

exerts no depressor effect and adrenalin but slight pressor effect. It is therefore evident that in these conditions of low pressure the injury to the peripheral vasomotor mechanism is one involving the nerve-endings rather than the muscle; the function of the nerve-endings, however, is not completely in abeyance.[13]

TREATMENT

From the foregoing discussion of the causation of low blood-pressure in "peptone" intoxication and anaphylactic "shock" it is evident that our therapeutic experiments have for their object the amelioration of a circulatory condition due to an action on the peripheral vasomotor apparatus and characterized by an excessive accumulation of blood in the large venous trunks of the abdomen. There is, however, this difference in the character of the low pressure due to "peptone" and that due to anaphylaxis, which must be clearly recognized in considering the results of therapeusis. Although the anaphylactic condition may be of short duration, it is usually characterized by a low circulatory condition which frequently persists for long periods of time without change and is closely analogous to the condition of shock as generally known. All changes due to treatment under such circumstances are therefore to be ascribed directly to the action of the agent employed, with but slight tendency to immediate spontaneous recuperation on the part of the animal. On the other hand, the low blood-pressure of peptone intoxication is maintained in the average intoxications for only five to six minutes, with, after that time, a gradual tendency to improvement. It resembles a condition of collapse rather than shock. Under such circumstances all treatment after the first few minutes must be considered as aiding the natural recuperative power of the temporarily embarrassed vasomotor system.

TREATMENT BY TRANSFUSION

The condition of the general circulation following an increase in blood-volume by transfusion, after some of the experimental procedures directed against the different divisions of the vasomotor system, serves to illustrate the importance of the rôle played by these separate divisions in the process of recovery. When a dog is decapitated and transfused, the blood-pressure rises rapidly in proportion to the duration of transfusion and is well maintained. When, however, in addition to decapitation, the spinal cord is mechanically destroyed, transfusion causes a delayed and inadequate rise that is poorly sustained. With the loss of tonic con-

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13. The experiments described were carried out on both types of low pressure and also on animals with the peptone effect added to that of anaphylaxis. The results were the same.

strictor impulses from the spinal centers, a passive dilatation of the vessels occurs and the small, slow rise in pressure obtained with even a copious transfusion does not appear until the vessels are practically distended to their full limit and is, presumably, purely a mechanical feature. Before a level is reached which is comparable to the level obtained by a similar transfusion in a decapitated animal, the distention and loss of elasticity of the vessels is so great that the heart, which is receiving an insufficient blood-supply, is unable to increase the arterial pressure sufficiently to relieve the venous congestion.

The condition of the circulation in anaphylactic shock[14] is in a way analogous to that of an animal with spinal cord and medullary centers destroyed. Both centers exert little or no influence on the peripheral mechanism owing to the blocking at the neuromuscular junction, and the regulatory function of the medullary centers is further influenced by the local anemia consequent on general low pressure. Consequently venous transfusion increases the dilatation of the peripheral circulation and causes an increased congestion of the splanchnic area, without materially augmenting the amount of blood going to the heart and medulla. If we compare the circulatory condition of anaphylactic shock with that of hemorrhage, we find in both a common condition—anemia of the medullary centers—but widely different conditions in the peripheral mechanism. In anaphylactic shock the latter is out of function, while in hemorrhage its regulatory power is intact, and thus is maintained a fairly efficient circulation supplying the heart and medulla, and venous transfusion under such circumstances causes a rapid return to normal. In anaphylactic low pressure, on the other hand, such transfusion increases the splanchnic congestion without aiding the heart or the medulla. It is evident, therefore, that the two conditions to be combated are (1) the congestion of the splanchnic area and (2) the anemia of the heart and medulla. As preliminary experiments showed that drugs have little or no power to overcome both conditions, we resorted to arterial transfusion to relieve the second, and in some experiments, have bled simultaneously from the central end of the femoral veins, to reduce the splanchnic congestion.

This combination has been most successful. Bleeding from the femoral vein removes from the great veins of the abdomen a large amount of blood containing accumulated toxic products and also allows the relatively fresh blood of arterial transfusion to come in contact with the splanchnic vessels, thus presumably hastening the recovery of the per-

14. The peptone intoxication, although analogous, is not strictly comparable on account of the tendency to gradual spontaneous recovery.

ipheral vasomotor mechanism. At the same time the transfusion (by carotid anastomosis) brings a large volume of fresh arterial blood directly to the capillaries of the medulla and the heart,[15] and hastens their recovery before materially adding to the volume of the blood in the large venous trunks of the splanchnic area. This combination of venous bleeding and arterial transfusion is efficacious in both peptone intoxication and anaphylactic shock, and readily brings about a condition of stable equilibrium. On the other hand arterial transfusion, alone, of moderate degree, has been sufficient to bring about a restoration of pressure with maintenance at the original level, but the danger of over-transfusion and cardiac breakdown is greater.

These observations demonstrate conclusively the importance of the part played by the medullary centers in recovery from conditions of low blood-pressure.[16] This has been well illustrated in the work with an isolated cerebral circulation, when, with these centers unaffected by the depressing agent, and having a constant independent blood-supply, the duration of low blood-pressure, following the intravenous injection into the trunk of peptone or the anaphylactic dose of horse-serum, was markedly decreased and the recovery was more rapid than is the case in the intact animal.

These results, successful as they are in experimental procedure, can hardly be recommended as a basis for similar therapeutic measures in treating low blood-pressure in man. They do, however, offer a very satisfactory basis for the experimental study of the influence of drugs and other remedial agents.

TREATMENT BY DRUGS

Since in peptone intoxication the blood-pressure tends to rise spontaneously after ten to fifteen minutes, even when large doses are given, the attempts to overcome the effects of peptone by therapeutic measures have been confined to the period when spontaneous recovery was not to be expected. We have assumed that if within a period of five minutes after the production of peptone intoxication the blood-pressure could be raised and sustained at the normal level throughout the usual period of

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15. Anastomosis of a carotid of the donor to the central end of a carotid of the recipient allows, on account of the low pressure in the latter, the direct passage of blood not only to the brain of the recipient through the other carotid, but also to the coronary arteries, thus directly supplying the capillaries of the heart muscle.

16. Crile, G., and Dolley, D. H.: On the Effect of Complete Anemia of the Central Nervous System in Dogs Resuscitated After Relative Death, Jour. Exper. Med., 1908, x, 782.

peptone action, a satisfactory line of treatment for this and analogous clinical conditions would be indicated.

With this end in view we have employed normal saline solution, adrenalin, caffein, digitoxin and strophanthin. These were administered through a cannula in the saphenous vein, with due regard to the temperature of the solutions and the rate of injection.

It has been found that saline solution has its usual effect, proportionate to the amount injected, of mechanically raising the pressure which, however, is not well sustained owing to the rapid passage of the fluid into the tissues. The administration of cardiac stimulants simultaneously while causing increased heart action in the usual degree and a still further rise in pressure, do not suffice to offset the loss of the mechanical effect of the saline.

Adrenalin in therapeutic doses in normal saline solution causes the most satisfactory rise in the blood-pressure and, with it, a heart action quite comparable to that obtained before the administration of the peptone. This improvement, however, is limited by the duration of the adrenalin action. Again, the simultaneous administration of cardiac stimulants causes a further rise and a more prolonged effect, but, as the constrictor effect of the adrenalin wears off, the blood-pressure again returns to a low level. During the maximal peptone action the rise of blood-pressure following adrenalin administration is small and transient even on a percentage basis. But if such administration is made late in the peptone action when the vasomotor endings are recovering and the condition of the medullary centers is likewise improving, even though the pressure is still low, the rise is proportionately greater, and subsequently the general pressure is sustained at a normal level.

The response to cardiac stimulation, in so far as the heart itself is concerned, is quite as active and as well within the normal limits as before the administration of peptone, but owing to the peripheral dilatation and the diminished volume of blood reaching the heart, the slight improvement in the general blood-pressure which ensues is quite transient. The heart shows the effect of overaction due to insufficient blood-supply, its contractions weaken and the blood-pressure quickly returns to or below the former level. If, however, as with adrenalin, such treatment is instituted toward the end of the peptone action, the recovery is much hastened and the heightened blood-pressure, due to the increased heart action, is well sustained.

While we are unable to directly neutralize the effect of the peptone intoxication or to activate the peripheral vasomotor mechanism, and are convinced of the futility of attempting to combat the condition by cardiac

stimulants alone, the results obtained by the employment of a slow, continuous injection of adrenalin in salt solution (1-40,000), administered intravenously, with the addition of a pure cardiac stimulant such as digitoxin, thus promoting the determination of blood to the right heart and an increased circulation in the brain, lead to the conclusion that a similar line of treatment is indicated in those clinical emergencies that present an analogous circulatory condition. Such treatment is not, however, specific or curative, except in so far as it serves to tide over successfully the critical period, until the effects of the toxic agent have worn off and the normal functions of the vasomotor mechanism are resumed. This has been satisfactorily demonstrated in the more prolonged condition of anaphylactic shock in which, as during the early-stage of peptone intoxication, adrenalin has a minimal effect, and in which, likewise, cardiac stimulants alone are unable to overcome the effects of the paralysis of the vasomotor nerve-endings. On the other hand, a combination of the peripheral constrictor effect of a slow continuous injection of adrenalin, of salt solution and of an active cardiac stimulant, brings the circulation within the normal limits long before spontaneous recovery could be expected to occur, and maintains it at this level.

SUMMARY

Anaphylactic shock and peptone intoxication are characterized by conditions of low blood-pressure very similar to those seen in shock and collapse, and, as they are produced without trauma or other factors usually concerned in the etiology of surgical shock, are of interest in the study of conditions of shock occurring in medical as contrasted with surgical practice.

Both conditions are characterized by a fall in blood-pressure to a level of 20 to 30 mm. Hg, which is prolonged in anaphylactic shock, but tends to relatively rapid recovery in peptone intoxication. In both conditions there is an extreme congestion of the large venous trunks of the splanchnic area with a coincident medullary anemia. The respiration is not altered except in as far as it is affected by the anemia of the medullary centers; the heart shows no initial changes, the low pulse pressure being due apparently to the small amount of blood passing through it.

Physiological studies having for their object the determination of the mechanism by which the low pressure is caused, demonstrate that the condition is essentially a peripheral vasomotor paralysis. Pharmacological studies indicate that the effect is on the nerve-endings rather than on the muscle.

With independent cerebral transfusion the recovery from low pressure is more rapid than in the intact animal. This is true also when an animal is transfused by carotid anastomosis, and recovery is especially satisfactory when the transfusion is accompanied by simultaneous bleeding from the femoral vein.

The indications for treatment, therefore, appear to be (1) relief of splanchnic congestion and (2) increase of volume of blood to the heart and medulla. Cardiac stimulants alone, or salt solution or adrenalin alone, cannot bring about a permanent improvement. A combination, however, of the slow injection of adrenalin in salt solution (1 to 40,000) intravenously with the addition of a pure cardiac stimulant, as digitoxin, leads to relatively rapid and permanent improvement, by promoting a determination of the blood to the right heart and increasing the circulation in the brain.

The results of the experimental treatment of this condition, which, for want of a better name, may be called toxic shock, are not essentially different from those in traumatic shock.

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EXPERIMENTAL POLIOMYELITIS IN MONKEYS

EIGHTH NOTE: FURTHER CONTRIBUTIONS TO THE SUB-
JECTS OF IMMUNIZATION AND SERUM THERAPY *

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In our seventh note[1] on experimental poliomyelitis we treated of some of the phenomena of active immunization and passive serum protection in respect to epidemic poliomyelitis, and in this connection we wish to record some additional observations of the same general import. It can now be accepted as established that human beings and monkeys who have passed through an attack of poliomyelitis have come to contain in their blood certain neutralizing principles for the virus of poliomyelitis; that these principles are readily demonstrable by animal tests for two or more years in human beings, and that they probably persist for as great a period in monkeys. It has, moreover, been shown that monkeys which have recovered from an attack of poliomyelitis are highly refractory to reinoculation with the virus of poliomyelitis, and it is probable that the recovered human beings are similarly protected from reinfection. In both instances the attack has conferred a strong active immunity. But to have had and to have recovered from poliomyelitis is not the sole way in which an active immunity can be achieved, since it has been proved possible to immune monkeys actively by injecting into the subcutaneous tissue either the virus of full strength or one modified by chemical agents (for instance, glycerin).

ARTIFICIAL ACTIVE IMMUNIZATION

This mode of producing active immunity has not up to the present time been developed into a uniformly successful and safe method, since of the treated animals some do not develop a strong immunity and others develop paralysis as a result of the treatment.

The test of this active immunity thus far published consisted of the ability to resist a large intracerebral injection of the filtrate that in far smaller quantities produces paralysis in the control animals. The active immunity has been secured either from a single large subcutaneous injection of the crude or modified virus as represented by emulsions of the spinal cord taken from recently paralyzed monkeys, or by repeated injections of gradually increased amounts of the crude virus.

SERUM NEUTRALIZATION OF THE VIRUS

It has been shown that the blood-serum of human beings and monkeys that have recovered from an attack of poliomyelitis contains neutralizing principles for the virus of the disease, which principles are absent from normal serum. Moreover, it is safe to assume that these neutralizing substances are the indication and cause of the active immunity which is responsible for the refractory state of the animals to reinfection. Hence there should exist in the serum of the directly actively immunized animals, which have not been made to pass through an attack of poliomyelitis, similar neutralizing principles. When an active filtrate containing virus is mixed with the serum of actively immunized monkeys and incubated for a period at 37 C. it no longer sets up paralysis on being injected into the brain of normal monkeys.

In the previous neutralization experiments with monkey serum reported, the serum had been taken from animals in which, after recovery, reinforcement of the immunity had been attempted either purposely by subsequent injections of considerable quantities of active virus or had been circumstantially produced in the course of tests made to determine the existence of immunity to reinfection. We have since ascertained that without reinforcement the serum of monkeys that have recovered from the paralysis contains readily appreciable quantities of this principle. This experiment has established the fact that monkeys react precisely as human beings do in respect to the disease, since their serum as such after recovery possesses marked neutralizing power for the virus *in vitro;* and it indicates that the test would probably disclose the fact of an atypical attack of poliomyelitis such as is embraced in the so-called abortive type of the affection.[2]

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2. Netter and Levaditi have indeed determined that the blood of a patient who had suffered an abortive attack of poliomyelitis contained the neutralizing principle.

EXPERIMENTAL SERUM THERAPY WITH MONKEY SERUM

In the seventh note we pointed out that the serum derived from immune monkeys that had passed through an attack of poliomyelitis possessed therapeutic properties for the experimental disease. It was stated that if the quantity of virus injected into the brain was not in excess of a given dose the development of paralysis could, in some cases, be prevented by making several injections of the serum, by lumbar puncture, into the subarachnoid space, and that infection by way of the nasal mucosa could also and with greater certainty be prevented by the serum injections. We have also ascertained that the scrum of directly immunized monkeys possesses similar therapeutic properties.

EXPERIMENTAL SERUM THERAPY WITH HUMAN SERUM

That human serum derived from children who have passed through an attack of poliomyelitis possesses neutralizing power for the virus of poliomyelitis has been stated already; it can now be stated that it possesses therapeutic value also. When the subarachnoid injections are begun twenty-four hours after the intracerebral inoculation of the virus, the development of paralysis can be entirely prevented in a certain number of the animals, while in another number the onset of paralysis is much delayed. ·

The period of incubation in the control animals ranged from seven to eleven days and in some of the treated animals that finally became paralyzed it was as great as twenty-six and twenty-seven days. The serum injection begun twenty-four hours after the inoculation, was carried out on three successive days, after which an interval of three days was allowed to pass, when three more daily injections were given. It seems probable that had the injections been resumed after another interval of rest the last vestige of the virus in the tissues would have been destroyed.

NEUTRALIZED VIRUS PRODUCES NO IMMUNITY

We have seen that actively immunized monkeys, the result of obvious disease and paralysis, or the product of gradual or abrupt accommodation to the virus, not only yield a serum that contains neutralizing properties for the virus, but are themselves highly refractory to reinfection with a highly potent virus. The fact has been repeatedly observed that failure to develop paralysis after an intracerebral inoculation of an active virus

does not lead to any increase in resistance of the monkeys to subsequent infection by the same route. The animals which have once withstood an inoculation respond on another occasion as readily as the control animals. No explanation for this discrepancy has been found.

It is of interest, therefore, to learn that monkeys which have been protected from paralysis through the employment of mixtures of virus and immune serums, or as result of treatment by subarachnoid injections of immune serums, do not exhibit any unusual degree of resistance to subsequent intracerebral injections of active virus made at periods of several weeks to four or five months after the condition of the original experiments. Three important points are developed by this fact: that neutralized mixtures of virus and immune serum do not lead to any degree of active immunization; that the therapeutic action of the serum is associated with restraint of multiplication of the virus such as would be required to establish any grade of active immunity; and that a simple passive immunity is either not produced by the serum injections, or is of brief duration or small amount.

ARTIFICIAL IMMUNE SERUM

We have continued our efforts to produce an immune serum in some of the lower animals that might possibly become the source of a therapeutic serum. This work is being actively pursued at present, but we wish to report on an indication in the direction of our search. It has been found that normal sheep serum possesses a definite although slight neutralizing power for the filtered virus, and that the injection of emulsions of the spinal cord and brain of recently paralyzed monkeys into the sheep augments this property of neutralization.

The serum treatment of poliomyelitis is at present in the experimental state, and how soon, or whether ever at all, it will be applicable to the spontaneous disease in human beings cannot be predicted.

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THE PHYSIOLOGY OF ANAPHYLACTIC "SHOCK" IN THE DOG.*†

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THE investigation here presented was incidental to a study of the treatment of various experimental conditions of low blood pressure,[1] one of which was that associated with anaphylaxis in the dog. As a knowledge of the mechanism of the various forms of low pressure was essential to treatment, extensive physiological studies were made, and as the results of the study of anaphylactic shock are striking and have a very definite bearing on the problems of anaphylaxis in general, they are, therefore, made the subject of this communication.

Physiological and pharmacological studies of anaphylaxis are few in number and with the exception of the work of Arthus on the rabbit and Biedl and Kraus on the dog are limited to the guinea-pig. The general opinion[2] has been that the underlying cause is a change in the cells of the central nervous system and this view has had some support from the observations of Besredka, who found that the clinical manifestations of anaphylaxis could be lessened by ether anesthesia, and of Banzhaf and Famulener, who found a hypnotic dose of chloral hydrate would prevent symptoms in sensitized animals. The observations of these writers are, however, somewhat contradictory, and Rosenau and Anderson state that altho ether anesthesia may mask the symptoms, it does not prevent death.

Attempts to demonstrate specific anatomical lesions have been most unsatisfactory. Gay and Southard state that "we have to

deal with an intimate cell reaction, demonstrable by definite cell lesions." They describe hemorrhages in the gastric mucosa and fatty changes in voluntary muscle, heart muscle, and nerve fibres. These observations have been confirmed in detail by Tscharnotzky.[1] The respiratory disturbance has been especially studied, first by Gay and Southard and lately by Auer and Lewis. The former consider the cause of death to be respiratory; respiration ceases in the inspiratory phase with gross and microscopic evidence of emphysema. A striking feature is severe diaphragmatic spasms. Rosenau and Anderson state, in view of the persistence of the heart beat after failure of respiration, that death is due to an effect on the nervous control of respiration. Auer and Lewis[2] conclude that anaphylactic death is due to asphyxia produced apparently by a tetanic contraction of the smooth muscle of the bronchioles. This contraction gradually occludes the lumen of the bronchioles and finally no air enters or leaves the lung, and in spite of evident respiratory efforts, the animal is strangulated. The lungs are distended in an inspiratory position and do not collapse when the chest is opened. The condition is due to a peripheral action, for destruction of spinal cord and medulla in no way affects the occurrence of the condition of the lung. Auer and Lewis do not care to give an opinion as to whether the toxic dose exerts its effect on the bronchial muscles alone or upon the vagus motor endings, or both. They also describe an initial rise in blood pressure, with later a gradual drop to 10 to 20 mm. of mercury or less, within 10 minutes. Heart block, probably due to asphyxia, also develops. Auer and Lewis also make the interesting observation that the administration of atropin, which paralyzes the bronchial muscles, causes a relaxation of the rigid anaphylactic lung, and that if atropin is given before the toxic dose of horse serum the pulmonary symptoms may be greatly reduced or prevented entirely. Anderson and Schultz[3] confirm most of these observations, but find that the administration of chloral hydrate, urethrane, adrenalin, and pure oxygen also may prevent the death from asphyxia. When life is thus prolonged, death results from the condition of low blood pressure.

[1] "Travail de l'Institut Bact. de Moscow, 1909," *Réf. Bull. de l'Institut Pasteur*, 1909, 7, p. 591.

[2] *Jour. Amer. Med. Assoc.*, 1909. 53, p. 458; also *Jour. Exper. Med.*, 1910, 12, p. 151.

[3] *Proc. Soc. Exper. Biol. and Med.*, 1909 7, p. 32.

Arthus,[1] who has worked with the rabbit, describes a very striking fall in blood pressure with polypnea, involuntary evacuation of feces and suppression of urine.

Anaphylaxis in the dog has not been extensively studied. This is possibly due to the negative results of Remlinger, and in part also to the fact that the phenomena in the dog differ in some respects from those in the guinea-pig. The chief differences are (1) the absence in the dog of the respiratory disturbance so characteristic in the guinea-pig and (2) the occurrence in the former animal of a fall in blood pressure which is more striking than that in the guinea-pig. The fall in blood pressure in the dog is considered by Biedl and Kraus[2] who first described it, to be the essential phenomenon of anaphylactic shock and to be due to a primary peripheral vaso-dilatation. These conclusions we have confirmed in an earlier communication.[3]

That the phenomena following an injection of horse serum into dogs previously sensitized with the same serum are to be considered as manifestations of anaphylaxis is shown by the following observations:

1. The intravenous injection of horse serum into a normal dog produces no clinical symptoms and no change in blood pressure.

2.   An intravenous injection into a conscious animal three weeks after a subcutaneous injection of the same serum produces the following clinical manifestations:

Dog 50, weighing 4,680 gm., received 5 c.c. of normal horse serum subcutaneously on February 26. Twenty-three days later (March 21) 5 c.c. of the same serum were injected slowly into a superficial vein of the left hind leg under local anesthesia. Before the entire amount was injected, the animal became restless, and made retching movements. This was followed within two minutes by vomiting and involuntary evacuation of feces and urine. During this period, the animal when placed on the floor took a few steps, slowly and with a peculiar stiff-legged gait. At the end of three minutes it fell to the floor slowly and lay on its side with head prone. Retching movements continued. Respiration was somewhat deep and labored and 28 per minute as compared with 36 per minute before injection, no dyspnea; the pulse in the femoral artery could not be felt; superficial reflexes normal. Vomiting and involuntary defecation and micturition continued more or less intermittently for seven minutes after injection. The animal when placed upright could not stand, but this appeared to be due to muscular weakness rather than to paralysis; was indifferent to surroundings but entirely conscious. At the end of 22 minutes the pulse was palpable in the femoral; the animal could stand

[1] *Arch. Internat. d. Phys.*, 1909, 7, p. 471.

[2] *Wiener klin. Wchnschr.*, 1909, 22, p. 363.

[3] R. M. Pearce and A. B. Eisenbrey, *Proc. Soc. Exper. Biol. and Med.*, 1909, 7, p. 30.

alone but preferred to lie down; though 10 minutes later it got up an walked around but was still weak. This degree of recovery occurred about half an hour after injection, and after the lapse of an hour the respirations were 28 per minute, the pulse readily palpable, the animal was weak but had power of locomotion, slight diarrhea was present, and the dog refused food. On the following morning he was found dead, a period of less than 15 hours having elapsed since injection. The cage showed evidence of profuse bloody diarrhea.

Autopsy: No excess of fluid was found in the peritoneal and pleural cavities, the serous surfaces were smooth. The bladder contained a small amount of clear yellow urine. The intestines appeared somewhat dark in color and the lower 15 cm. of the rectum showed numerous pin head petechial spots beneath the peritoneum; larger ecchymotic areas occurred beneath the serosa of the gall bladder. Spleen, liver, and kidneys appeared normal. In the greater curvature of the stomach the mucosa over two areas of about 1.5 cm. in diameter was intensely hemorrhagic and swollen and some superficial erosion of the epithelium was evident. In the region of the pylorus both the stomach and duodenum were normal, but about 2 cm. below the pylorus and for a distance of 27 cm. along the small intestine, the mucosa was greatly swollen and hemorrhagic. Below this area the Peyer's patches were elevated and dark colored, but hemorrhages were absent. A few centimeters below the ileocaecal valve the colon was intensely hemorrhagic with considerable erosion of epithelium. The mucosa of the gall bladder was smooth and showed no hemorrhage. Heart and lungs appeared normal; the latter collapsed on opening the thorax and presented some hypostatic congestion in dependent parts. The brain showed no macroscopic lesions.

3. In animals under complete ether anesthesia the objective manifestations of anaphylactic shock are absent;[1] in all such, however, an immediate and prolonged fall in blood pressure which persists for a considerable time occurs. This has been seen in 40 of 42 animals; in two dogs with pneumonia (distemper) it failed to occur. The lowered pressure is unaccompanied by noteworthy respiratory or cardiac disturbance.

4. After anaphylactic shock has been produced in a sensitized animal and the animal has recovered from the acute manifestations, subsequent injections fail to cause a reaction evident either by clinical symptoms or by change in the blood pressure.

These results, which are in accord with those of Biedl and Kraus, indicate that the principles underlying anaphylaxis in the dog are the same as those for anaphylaxis in the guinea-pig.

We have regarded the fall in blood pressure as the constant and most characteristic phenomenon of anaphylactic shock in the dog, and have based our conclusions concerning the physiology of anaphylaxis on its presence or absence under various experimental conditions.

[1] Vomiting, involuntary defecation and passage of urine, have been observed once in an animal anesthetized by Gréhant's method.

The first experiments had for their object the determination as to whether the condition of low blood pressure was due to changes in the blood caused by the injection of the toxic dose or to changes in the fixt cells. It has been generally assumed in all recent investigations with the guinea-pig that the latter explanation is the correct one, but as far as we are aware this has never been determined by transfusion experiments such as we employed. Furthermore, Biedl and Kraus show that not only is there a change in the numerical relation of the two types of cells of the blood one to another, but also, and this we have confirmed, that a decreased coagubility of the blood occurs. It seemed important, therefore, to rule out at once the influence of possible primary or secondary toxic bodies formed in the circulating blood by the union of the bodies in the toxic dose of serum with those bodies which might be formed in the blood of the animal by the first injection.

Our procedure has been to exsanguinate, under ether anesthesia, a small normal dog (A) and to transfuse this animal by Crile's method with the blood of a larger sensitized dog (B) until the blood pressure reached approximately its original level. After sufficient blood has been obtained from B to raise the pressure of A, the sensitized dog is then bled to exsanguination and transfused from a third normal dog (C) until its pressure reaches its previous normal level. At the proper moment, the normal dog containing the blood of the sensitized dog, and the latter containing the blood of the normal dog, each receives intravenously the toxic dose of horse serum. In the former, a fall in pressure does not occur and in the latter it does, thus proving that the phenomenon of anaphylaxis is due to a reaction in the fixt cells and not either primarily or secondarily to changes in the blood. This result is in accord with the contention of Gay and Southard and of Friedberger that the reaction of intoxication in the guinea-pig is located within the body cells and is opposed to the theory of "antibodies."[1]

The following protocol, presented briefly, is illustrative:

Dog A, weight 6,260 gm., ether anesthesia, cannula in femoral artery, connected with mercury manometer and recording on a kymograph. Original pressure 92 mm. Bled from the carotid artery until no more blood flowed, the pressure dropping to

[1] Compare with views of Friedberger, *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, 1909, 2, p. 208; 1909, 3, p. 581; 1910, 4, p. 612, and Friedemann, *ibid.*, 1909, 2, p. 591.

16 mm. Hg.   Transfused from B for 17 minutes by carotid anastomosis, until pressure reached original level, when 5 c.c. of normal horse serum were injected into the femoral vein.   No change in pressure except slight mechanical rise.

Dog B (No. 17), weight 8,020 gm.   Had received 5 c.c. horse serum subcutaneously 25 days before.   Ether anesthesia.   Arrangement of cannula as in A.   Original blood pressure 90 mm.   After transfusion sufficient to raise pressure of A, animal B was bled for 16 minutes until flow ceased, when blood pressure was at a level of 14 mm.   Transfusion from dog C was then begun and continued for 24 minutes when permanent level equal to previous normal level was obtained.   At this time 5 c.c. horse serum were injected into the femoral vein with an immediate fall in pressure to 24 mm Hg.

Dog C, normal dog, weighing 9,100 gm., prepared as were A and B, and used for transfusion of B.

Having thus determined that the reaction was evidently due to an effect on the fixt cells of the body and presumably on those of the vasomotor system, the question of the general distribution of the blood in anaphylactic shock was studied as a preliminary to the study of peripheral and central influence.   For this purpose, experiments were made under the following conditions:

All animals were under full ether anesthesia.   The blood pressure was taken from the left femoral artery with a mercury manometer. Injections were made into the right saphenous vein.   The changes in blood distribution were determined by oncometric studies by means of guttapercha capsules applied to spleen, kidney, and intestine, by a cannula introduced into the right common iliac vein and projecting into the inferior vena cava, by plethysmographic study of a forelimb and by a metal cylinder penetrating into the cranial cavity. Each of these was connected by rubber tubing with a bellows recorder allowing simultaneous records on a revolving drum.   It was thus possible in a single experiment to obtain simultaneously, as was frequently done, records of volumetric changes in the femoral artery, iliac vein, kidney, spleen, and intestine, with also a record of the respiration, or any combination of these with a record of variation in a limb or in intracranial pressure.

The temperature of the animal was maintained by the use of a hotwater coil encircling the greater part of the trunk and a record of changes in temperature was obtained by a thermometer in the rectum.   Normal horse serum was used in doses of 5 c.c., subcutaneously for sensitization, and the physiological experiment was made after about 21 days.   The dose used to bring about anaphylac-

tic "shock," usually 5 c.c., has varied from 2 to 6 c.c., altho it must be admitted that no attempt has been made to determine the minimal toxic dose. It has always been given intravenously.

The study of blood distribution included six observations. The fall in arterial pressure, as well as the change in the volume of the kidney, and the character of the respiration, are shown in Fig. 1.

It is apparent that anaphylactic shock is characterized by an abrupt fall of blood pressure averaging 50 to 70 mm. Hg which is independent of initial change in heart action, tho during the continuance of low pressure the amplitude of the pulse wave is greatly diminished, the result presumably of the small amount of blood reaching the heart. Respiratory disturbances, except in as far as they occur as a result of medullary anemia due to the low general arterial pressure, are absent. Oncometric studies show a decrease in the volume of kidney, intestine, and spleen simultaneously with the decrease in arterial pressure. Of these three organs the decrease is, as a rule, most marked in the kidney and least in the intestine. A very slight initial decrease in brain volume has been found as well as a slight diminution in the volume of an extremity. This decreased peripheral circulation is accompanied by an accumulation of blood in the liver and large veins of the abdomen. This has been determined by inspection of the liver and large veins and by the observation that a cannula introduced into the inferior vena cava and connected with a water manometer shows a moderate increase of pressure, equal to 6 to 10 mm. water, at the time of the fall in arterial pressure, with an almost immediate return to normal level as the blood becomes evenly distributed throughout the large venous trunks.

It is evident, therefore, that the important feature of the condition of low pressure here described is a lack of tone of the vessels, particularly of the splanchnic area, characterized by extreme venous congestion from which the animal does not quickly recover. It is essentially the condition characterized as a "bleeding into the veins of the abdomen" and in many respects is analogous to the circulatory disturbance of surgical shock.

The next step, naturally, was to determine to what extent this condition was due to peripheral and to what extent to central vasomotor disturbances. To determine the influence of the central
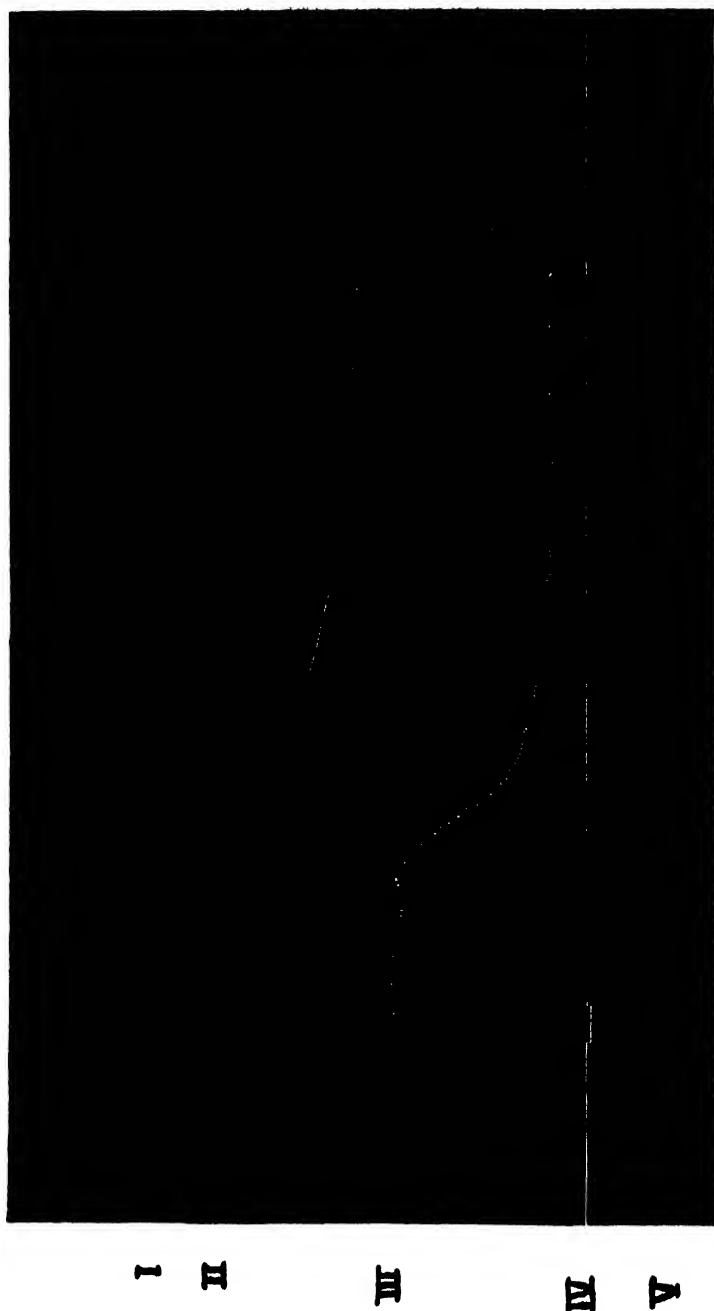
FIG. 1.—This tracing illustrates the effects of the injection of 5 c.c. normal horse serum into the saphenous vein of a dog (weight 838 ograms, that had been given 5 c.c. of the same serum 22 days before Ether anesthesia. I—respiration; II—kidney volume (oncometer on left kidney); III—blood pressure; IV—base line with signal at time of injection; V—time in seconds.

mechanism, experiments were done under ether anesthesia, in which the spinal cord, vagi, cervical sympathetic and splanchnic nerves were severed. None of these procedures, singly or collectively, prevented the fall in pressure, thus pointing to the absence or slight importance of central influence. Such evidence, tho usually considered conclusive by physiologists, we preferred not to accept without absolutely excluding the influence of centers in the cord as well as the secondary effect of cerebro-medullary anemia. Three groups of experiments were therefore performed. These included the study of anaphylaxis under the following conditions: (1) decapitation, (2) decapitation and destruction of the cord with blood pressure reinforced by transfusion, and (3) with the cerebral circulation independent of the circulation of the body. All experiments of these three groups were done under complete ether anesthesia by tracheal cannula.

1. The object of decapitation[1] was naturally to eliminate all cerebro-medullary influence. After this procedure, it was found that the blood pressure falls to a point about one-half of its original level and remains there with little variation, if care is exercised to keep the artificial respiration uniform and to maintain the body heat, for a period covering the usual duration of the experiments. With the loss of vagus influence the heart rate is quickened and the excursions of the pulse wave shortened. The respiratory curve in the blood pressure tracing is well marked. With these conditions prevailing the toxic dose of horse serum caused a fall of blood pressure to the level occuring from similar doses in intact animals. The onset of the fall was less abrupt and the low level was reached more slowly. That the fall could occur, however, independently of cerebro-medullary influence was clearly shown.

2. In the experiments in which the cord was mechanically destroyed after decapitation, the object was to eliminate all except local vasomotor influences. After destruction of the cord it was found that a further dilatation and fall in blood pressure occurs beyond that caused by the decapitation and that death rapidly ensues. This is apparently due to the fact that with the additional loss of the spinal con-

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[1] Decapitation may be performed with a comparatively negligible amount of hemorrhage and therefore with but slight effect on the circulation of the trunk, by clamping the common carotid arteries and the internal and external jugular veins simultaneously low in the neck, having first ligated the vertebral arteries at the point where they enter their canals in the transverse processes of the cervical vertebrae.

strictor influence an insufficient volume of blood reaches the heart. By means of transfusion, however, it was possible to raise the blood pressure and maintain the circulation at a point permitting mano-metric record of further change in the pressure, and under such circumstances it is found that the injection of horse serum into a sensitized dog causes a definite fall in pressure, demonstrating conclusively that the essential phenomenon of anaphylaxis may be brought about independently of the cerebro-medullary and the spinal centers.

3. While the experiments on decapitated animals and on those with spinal cord destroyed and with vagi and cervical sympathetic nerves severed have shown that the usual effect on the blood pressure may be produced independently of the central action, such experiments have not of course shown that the central vasomotor mechanism does not perhaps play some part in the symptom complex presented by intact animals.

With the object of ascertaining the possibility of a central action in anaphylaxis, without the complication of the known peripheral effects, we were obliged to devise a means by which the horse serum might be introduced into the cerebral circulation, but not reach the circulation of the body. For this purpose a special operation was perfected, the details of which are described elsewhere,[1] whereby all vascular communication between the trunk and head was obliterated and the circulation in the head and neck maintained independently by transfusion from the carotid artery of a normal animal, the outflow from the recipient being through a free opening in the external jugular vein. Following a short period of irregularity due to the temporary changes in the cerebral circulation incidental to the technic of isolation, the general blood pressure of the recipient is maintained at a normal level, and horse serum may be introduced into the cerebral or into the peripheral circulations exclusively without any of the injected material reaching the circulation of the other portion of the animal.

When, under such circumstances, horse serum is introduced through the anastomosing carotid artery into the cerebral circulation of a sensitized animal, a slight transient lowering of the blood pressure is produced, but it is not the typical depression of anaphy-

[1] A. B. Eisenbrey, *Proc Soc. Exper. Biol.*, 1910, 7 (in press).

lactic shock in intact animals. The action is prompt, but the depression, to give the figures in one experiment, is only 16 mm. Hg with return to normal level in 23 seconds. If, some minutes after recovery from this effect, the serum is injected into the circulation of the trunk, it produces a lowering of the blood pressure to a level which corresponds to that in the typical condition of anaphylactic shock in the intact animal, for example, in the experiment quoted a fall of 74 mm. Hg with the low level persisting.

In view of the well-known fact that when anaphylactic shock is once produced further injections of the serum have no effect, the results in the above experiments show not only the completeness of the isolation of the cerebral circulation from that of the trunk but also that the peripheral influence is the essential factor in the production of the change in blood pressure so characteristic of anaphylaxis.

It was apparent, therefore, that the action was in the largest degree peripheral, either on the nerve endings or on the muscle of the vessels themselves, which is in accord with the observations of Biedl and Kraus concerning the influence of barium chloride in modifying the blood pressure phenomena of anaphylactic shock.

A number of pharmacological experiments were then made to still further localize the action. These are based on the experiments of Dixon[1] and Brodie and Dixon[2] with apocodeine. This substance in very large doses paralyzes vasomotor nerve endings; and "when its action is complete pilocarpine, physostigmine, and adrenalin have no effect on the blood vessels or blood pressure, whilst barium and digitalis will still constrict the blood vessels and raise blood pressure. The first three drugs, it is therefore assumed, act on nerve endings and the lat er two directly on muscle."

As the low pressure of anaphylaxis both in its level and in permanence resembles closely that due to apocodeine we have tested the effect of adrenalin and barium in the hope of finding analogous mechanisms. The results are not conclusive. While barium causes an increase in pressure,[1] adrenalin does likewise; the latter, however, is but slight and never equal, even if percentile values are considered,

[1] *Jour. of Physiol.*, 1903, 30, p. 97.   [2] *Ibid*, p. 476.

[3] We have also confirmed the observation of Biedl and Kraus that the administration of barium chloride to a sensitized animal before giving the toxic dose modifies or prevents the characteristic fall in pressure.

to the normal adrenalin reaction. Assuming therefore that adrenalin acts through the nerve endings, it is evident that in anaphylactic shock altho these structures are not completely paralyzed, their activity is greatly diminished not only as shown by the slight adrenalin action but also by the fact that splanchnic stimulation causes a smaller rise in pressure than in the normal animal. Nitroglycerin, which according to Dixon and Brodie acts only on the vessel muscle, also causes a further fall in pressure, as does also to a slight extent dog's urine, which as we have shown elsewhere,[2] apparently lowers blood pressure by an action on the nerve endings. It is therefore evident that altho this condition of low pressure is due in greatest part, at least, to a disturbance of the peripheral vasomotor mechanism, it is not possible to say that the function of either the nerve endings or the muscle is completely in abeyance. The pharmacological experiments indicate, however, that the nerve endings bear the brunt of the injury. This view is supported by experiments with apocodeine. If a sensitized dog under the influence of apocodeine received an injection of serum, a further depression of the circulation was not obtained. In fact, the blood pressure was raised a few millimeters by the mechanical effect of the injection and remained so for a period of about one minute, gradually returning to a point slightly below the original level after two minutes.

SUMMARY

1. Anaphylaxis in the dog is characterized subjectively by a sudden and persistent fall in blood pressure followed by objective symptomr referable to cerebral anemia.

2. Exsanguination and transfusion experiments demonstrate that the disturbance is due to a reaction in the fixt cells and not to changes occurring primarily or secondarily in the circulating blood.

3. Experiments in which the peripheral and central vasomotor mechanisms have been separated by various methods (section, decapitation, destruction of the cord, and isolated circulation) show that the chief influence is exerted on the peripheral vaso-motor system.

4. Pharmacological experiments point to an influence on the nerve endings rather than to one on the muscle of the vessels.

[2] *Amer Jour. of Physiol*, 1910, 26, p. 26.

THE INFLUENCE OF THE REMOVAL OF FRAGMENTS OF THE GASTRO–INTESTINAL TRACT ON THE CHARACTER OF NITROGEN METABOLISM. — III. THE EXCISION OF THE STOMACH.

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THE process of nutrition is accomplished in three distinct phases, those of digestion, of absorption, and of assimilation. Each of these contributes an equally important share in maintaining the integrity of the adult or permitting the growth of the young. The part played in this process by the different fragments of the digestive organs has been the subject of numerous investigations at all times. However, most of the efforts thus far were directed to the study of the first two phases. In recent years valuable contributions have been made regarding the laws governing these phases of the process and regarding the mechanism by which they are accomplished. It was found that the stomach played a subordinate rôle, both in the process of digestion and absorption, and on this ground there developed a tendency among physiologists to attribute to this organ a very secondary part also in the process of assimilation.

In a previous communication by Levin, Manson, and Levene,[1] on the nitrogen metabolism after gastroenterostomy, the observation was brought to light, that after this operation the power of the animals to retain and to assimilate the nitrogen of the food was diminished. The animals maintained their nitrogenous equilibrium, but all the nitrogen ingested in excess over the standard diet was removed within the first twenty-four hours following the intake. It should be added here that in the review of the literature on the subject given in that communication, several older publications regarding clinical observations on metabolism after gastroenterostomy in man were omitted.

[1] LEVIN, MANSON, and LEVENE: This journal, 1909-10, xxv, pp. 231–253.

They are those of Heinsheimer,[2] Joslin,[3] and Deganello.[4] The results obtained by these writers are in harmony with the view expressed in the first article of this series. They all contain records showing that after gastroenterostomy nitrogen retention is either totally absent or is very insignificant. It was realized at the time of that publication that more convincing information regarding the rôle of the stomach in the process of assimilation could be obtained through the study of protein metabolism on animals after complete gastrectomy.

It has been known since the time of Czerny and Kaiser[5] that man and animals may survive this operation and remain in apparently good health. However, there are recorded comparatively few investigations of the nitrogen metabolism after excision of the stomach. The few reported observations[6] were made on several persons operated for carcinoma. According to these reports patients after the extirpation of the stomach not only were capable of maintaining nitrogenous equilibrium but at times retained part of the ingested nitrogen. On the basis of these observations the authors reached the conclusion that normal metabolism is restored in man after gastrectomy. However, the conditions under which the work was done did not always permit of a desired thoroughness, and therefore the conclusions are not very convincing. Besides, it is necessary to bear in mind that the operation of gastrectomy was performed on individuals who suffered from a mechanical difficulty in receiving nourishment, and in whom the stomach was the focus of a diseased process and a source of intoxication. Thus the patients had been, for a long time previous to the operation, in a condition of under-nutrition, or even in a state of partial inanition. After the operation the patients are in a state of reconvalescence when the power of regeneration of the tissues is exaggerated. This consideration may serve to explain the peculiarity of the behavior of the patient under the observation of Deganello. On a diet containing 24.14 gm. of nitrogen, in four days the patient retained 3.03 gm. of nitrogen. This surely indicates a

[2] HEINSHEIMER, F.: Mittheilunge aus der Grenzgebieten der Medizin und Chirurgie, 1895, i, p. 348.

[3] JOSLIN: Berliner klinische Wochenschrift, 1897, p. 1047.

[4] DEGANELLO, N.: Archives italiennes de biologie, 1900, xxxiii, p. 132.

[5] CZERNY: Beiträge zur operativen Chirurgie, 1878.

[6] WROBLEWSKI: Centralblatt für Physiologie, 1897, xi, p. 21; HOFFMAN: Münchener medicinische Wochenschrift, 1898, p. 560; DEGANELLO: *Loc. cit.*

very unusual capacity for nitrogen retention. In considering the records of the patients with gastrectomy, it is also important to bear in mind that there exists no evidence that the removal of the stomach was complete.

The observations on animals as compared with those on men are not more satisfactory either in number or in character. Carvallo and Pachon[7] report a successful operation on a cat. The animal gained in weight immediately after the operation from 1,850 gm. to 2,255 gm. But, after some time, the condition of the animal grew very serious; it refused food and died six months after the operation. The observations of De Fillipi[8] on a dog are also very imperfect.

In the present investigation it was intended to follow the plan described in the two previous communications of this series,[9] but many difficulties presented themselves in placing the animals in nitrogenous equilibrium, in maintaining the uniform nitrogen output through all periods of the day, etc. Finally it was concluded to plan the principal experiments in periods lasting four or five days. Nevertheless, the catheterization of the animals was performed every twenty-four hours and the feeding took place at short intervals (every hour, ten times a day). Autopsies performed on the animals at the close of the experiments have revealed that in one of the animals a very small part of the pyloric end — not more than 15 mm. in length — still remained unexcised; in the second the resection was complete.

In the second animal at no time was there observed any nitrogen retention after the equilibrium was once established; in the first animal, immediately after the operation a marked retention of nitrogen could be observed, and only after some time, especially after the mode of feeding had been changed, the animal removed in twenty-four hours all the nitrogen taken in excess over the quantity required for the maintenances of the established equilibrium. An attempt to interpret the observations in detail will be given below; here it suffices to state that the results of the experiments on dogs with gastrectomy

[7] CARVALLO and PACHON: Archives de physiologie, 1895, vii, pp. 349–355 and pp. 766–770; also Travaux du laboratoire de Ch. Richet, 1895, iii, p. 456.

[8] DE FILLIPI : Deutsche medicinische Wochenschrift, 1894, p. 780.

[9] LEVIN, MANSON, and LEVENE: *Loc. cit.* and also CARREL, MEYER, and LEVENE: This journal, 1909–10, xxv, p. 439.

confirm the view previously advanced on the rôle of the stomach in the process of protein assimilation expressed in the first communication of this series.

PLAN OF EXPERIMENTS.

As soon as the animals recovered from the operation they were placed in a condition of nitrogen equilibrium on a diet containing not less than 70 calories per kilo weight. The food consisted of chopped lean beef, prepared according to the recommendation of W. J. Gies [10] and preserved in a refrigerator, together with plasmon, cracker meal, lard and salt. The attempt to maintain animals on the simpler diet of plasmon, cracker meal, and lard was abandoned, since it was noted that only in absolutely normal animals is this diet palatable for an indefinite time. All the animals that had undergone any one of the operations on the digestive organs took the simpler food only for a short time. This may be worth noting, since it contains perhaps an indication that even after an apparently complete recovery the animals in reality never again regained their normal health and the keenness of their normal appetite.

On the days of the experiments the animals received with the first meal, in addition to their standard diet, a quantity of plasmon equivalent to 1 or 2 gm. of nitrogen. The nitrogen elimination during that period was compared with the nitrogen output of a normal period of the same duration.

METHODS OF ANALYSIS.

The analytical methods employed in this investigation were the same as those described in the previous communication of this series.

EXPERIMENTS.

Dog I., weighing 13.5 kg., operated January 28th. Apparently uneventful recovery. On the 14th of February the animal vomited occasionally. This symptom did not recur again.

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[10] GIES, W. J.: This journal, 1901, v, p. 235.

Experiment I. — On the 28th of February the animal received a diet containing 5.89 gm. of nitrogen and 798 calories. The urine was collected in four-hour periods. On the following day the intake of the animal was raised to 7.82 gm. and 871 calories. Weight of dog, 12 kg.

The output on the two respective days was the following:

	Standard diet. Gm. N.	Standard diet + plasmon. Gm. N.
Intake	5.890	7.890
Output: feces	0.567	0.580
Absorbed	5.323	7.310
Output: urine	3.385	4.490
Balance	+1.938	+2.810

Absorbed in excess over first day . . .	1.987
Eliminated in urine over first day . . .	1.105
Balance between first and second day . =	+0.882

Thus there was noted a retention of 44.5 per cent of the excessive nitrogen intake. A normal dog under similar conditions[11] retained 43 per cent of the excessive intake.

Experiment II. — It is seen, however, that at the time of the experiment the animal was not yet in a condition of equilibrium. The diet was modified and on the 13th day of March the animal was in a condition of equilibrium at an intake of 3 gm. of nitrogen and 814 calories. Weight of dog, 12.06 kg.

On March 16 and 22 experiments were performed with a diet containing a quantity of plasmon equivalent to 1.0 gm. of nitrogen in excess over the standard diet. Weight of dog, 11.9 kg.

	Standard diet. Gm. N.	Standard diet + plasmon. Gm. N.
Intake	3.000	4.000
Output: feces	0.480	0.552
Absorbed	2.520	3.448
Output: urine	2.600	2.730
Balance	−0.080	+0.718

Absorbed in excess over first day	0.928
Eliminated in urine over first day	0.130
Balance between first and second day . .	+0.798

[11] LEVENE and MEYER: This journal, 1910, xxv, p. 217.

In these experiments there was noted a higher nitrogen retention than in the normal animal. This result seemed rather paradoxical at the first glance. A possible interpretation of it could be found in the following considerations. Under normal conditions the protein of food enters the duodenum only in form of primary digestion products, in small portions, thus bringing about a perfect digestion of the ingested protein material. After gastrectomy a large quantity of wholly undigested protein reaches the duodenum. The absence of gastric secretion may lead to a very imperfect secretion of the pancreatic juice and perhaps also of erepsin. Thus the protein material under these conditions remains in the intestinal tract for a considerable time undigested, and is absorbed in that form. The fate of such protein in the organism one would expect to be the same as that of parenterally introduced protein. It has been established by recent investigators that parenterally introduced protein is retained in the organism completely, if the animal is maintained in a condition of normal nutrition. The greater retention of nitrogen after gastrectomy, as compared with the retention after gastroenterostomy, observed on man, and in our experiments, may be interpreted in the light of these considerations. It was planned to subject this possible interpretation to further analysis. However, before sacrificing the animal, it was concluded to extend the observations, of the same character as described, to a longer period.

Experiment III. — On April 9 an experiment was begun lasting fifteen days. It was divided into three periods of five days each. During the first five days the intake of the animal contained 3 gm. of nitrogen and 814 calories. The following five days the intake contained 4.0 gm. of nitrogen and 846 calories, and the intake of the last five days was the same as the first period.

The results of this experiment were the following:

First period, st'd diet.		Second period, st'd diet + plasmon		Third period, st'd diet.	
Urine N.	Feces N.	Urine N.	Feces N.	Urine N.	Feces N.
2.40	.478	3.06	.540	2.90	.110
2.16	...	3.41	.596	3.28	.354
2.31	.901	3.75	.619	2.55	.717
2.73	.730	3.62	.583	2.76	.518
2.54	.540	3.40	.600	2.60	.463
Totals 12.14	2.649	17.24	2.938	14.09	2.162

	First period.	Second period.	Third period.
Intake	15.000	20.000	15.000
Output: feces.	2.650	2.938	2.162
Absorbed	12.350	17.062	12.838
Output: urine	12.140	17.240	14.090
Balance	+.210	−.178	−1.252

Absorbed in excess over first period . . 4.712
Output in urine over first period . . . 5.100
Balance over first period . . . . . −.388

Thus at this time no retention of the additional nitrogen took place.

Experiment IV. — Another experiment of the same nature was performed beginning April 30. The observations were made during eight days. They consisted of two periods. During the first, the intake of the animal contained 3.0 gm. of nitrogen and 814 calories. During the second, 4.0 gm. of nitrogen and 846 calories.

The result of this experiment was as follows:

	Standard diet.		St'd diet + plasmon.	
	Urine N.	Feces N.	Urine N.	Feces N.
	2.52	.418	2.94	.458
	2.40	.410	3.26	.436
	2.56	.561	3.16	.525
	2.49	.553	3.77	.757
Totals .	9.97	1.942	13.13	2.176

	First period.	Second period.
Intake	12.000	16.000
Output: feces	1.942	2.176
Absorbed	10.068	13.824
Output: urine	9.970	13.130
Balance	+.098	+.694

Again, in this experiment no nitrogen retention was observed during the period of additional nitrogen. It is also noteworthy that during the first two weeks following the operation the weight of the animal was continually increasing, while it remained constant after that period.

Thus it is possible that after a time the secretion of digestive glands is adapted to the new condition and the digestion of the ingested protein is completed in the intestines without the aid of gastric secretion. In this phase the animals find themselves in a condition similar to gastroenterostomy and the nitrogen retention becomes minimal. This interpretation seems very plausible. However, the autopsy of the animals revealed another condition which offers a basis for another interpretation for the difference in the fate of the additional nitrogen of the diet at the period immediately following the operation and at a considerably later period. Namely, it was noted on the autopsy that the upper part of the duodenum had undergone a considerable dilatation and its walls a marked hypertrophy. Thus it is possible that this artificial sack served as a mechanism regulating the transfer of the food to the intestines and in this manner rendering intestinal digestion more complete.

Dog II., weighing 14.36 kg., was operated on the 7th of January. Soon after the operation, the animal developed distemper. It slowly recovered health and on the 4th of April it was possible to begin the observations on the nitrogen metabolism of this animal. At that time the animal was in nitrogenous equilibrium.

Experiment I (April 4). — Food contained 5.29 gm. nitrogen and 756 calories; the weight of the animal was 11.1 gm. The following day the animal was given, in addition to the standard diet, a quantity of plasmon equivalent to 1.0 gm. of nitrogen, and on the day following this, again, the standard diet. The output in the three respective days was the following:

	First day. Gm. N.	Second day. Gm. N.	Third day. Gm. N.
Intake	5.29	6.29	5.29
Output: feces	0.66	0.69	1.33
Absorbed	4.63	5.60	3.96
Output: urine	4.72	5.68	4.49
Balance	—.09	—.08	—.53

Absorbed in excess over first day . . 0.97
Eliminated in urine over first day . . .96
Balance between first and second day +.01

Thus, in this experiment, all the additional nitrogen contained in the food of the second day was removed within twenty-four hours.

Experiment II. — This experiment lasted fifteen days. In course of the first five days, the animal received the standard diet of the first experiment; in the second period, also of five days, the animal received daily, in addition to the standard diet, a quantity of plasmon equivalent to 1 gm. of nitrogen, and during the third period the intake again consisted of the standard diet. The nitrogen balance during each of the three periods was as follows:

First period, st'd diet.		Second period, st'd diet + plasmon.		Third period, st'd diet.	
Urine N.	Feces N.	Urine N.	Feces N.	Urine N.	Feces N.
5.46	.469	6.52	1.010	5.55	.580
5.90	.990	6.32	1.165	6.08	.790
5.60	.546	6.13	1.085	6.10	.661
5.80	.840	6.34	.745	6.00	.432
5.50	.839	6.49	1.176	6.00	.728
Totals 28.26	3.684	31.80	5.181	29.73	3.191

	First period. Gm. N.	Second period. Gm. N.	Third period. Gm. N.
Intake	26.450	31.45	26.45
Output: feces	3.684	5.181	3.19
Absorbed	22.766	26.319	23.26
Output: urine	28.260	31.800	29.73
Balance	−5.494	−5.481	−6.47

Absorbed in excess over first period · · 3.553
Eliminated in urine in excess over first period 3.540
Balance . . . . +0.013

Thus also in this experiment no retention of nitrogen was observed. It was also noted that notwithstanding the intake to maintain the equilibrium of a normal animal of the same weight, this animal was losing both nitrogen and weight.

Experiment III. — This experiment was performed in order to test the correctness of the assumption that the very high rate of nitrogen retention observed in the first dog in the experiments performed soon after the operation was occasioned by the absorption into the circulation of the unchanged protein.

The animal at the time was receiving a diet containing 5.29 gm. of

nitrogen and 900 calories. On the 29th and 30th of April the animal
received a subcutaneous injection of 191 c.c. horse serum containing
3.09 gm. of nitrogen. The serum had been previously heated to 60° C.
for thirty minutes. The nitrogen balance two days previous to the
injection and on the day of the injection was the following:

	St'd diet.	St'd diet + horse serum 111 c.c. − 1.21 gm. N. subcutaneously.
Intake	5.29	6.50
Output: feces	.607	.624
Absorbed	4.683	5.876
Output: urine	6.050	6.25
Balance	−1.367	−.374

Thus all the nitrogen received by the animal parenterally was re-
tained in the organism in the same manner as the nitrogen of the addi-
tional diet in the early experiments on the first dog.

Experiment IV. — This experiment lasted six days and consisted of two
three-day periods. During the first period the diet contained 5.29 gm.
of nitrogen and 900 calories; during the second period it contained addi-
tional plasmon equivalent to 1.0 gm. of nitrogen, The nitrogen balance
of the two periods was the following:

First period, standard diet.		Second period, st'd diet + plasmon.	
Urine N.	Feces N.	Urine N.	Feces N.
4.80	.410	5.87	.539
4.80	. . .	5.64	.369
4.90	.815	5.85	.486
Totals . . . 14.50	1.225	17.36	1.494

	First period.	Second period.
Intake	15.870	18.870
Output: feces	1.225	1.494
Absorbed	14.645	17.376
Output: urine	14.500	17.360
Balance	+0.145	+.016

Thus in the three experiments on the second dog no nitrogen reten-
tion was observed. The fate of the additional nitrogen intake in this

animal was the same as in the dogs with gastroenterostomy, and the same as in the later experiments on the first animal with gastrectomy. In order to interpret the divergence in the early experiments on the first and the second animals it must be borne in mind that the observations on the second animal were begun on the twelfth week after operation; that in the first animal, in the experiments performed at the lapse of only ten weeks after the operation, already no nitrogen retention was observed after the additional intake. On the other hand, the autopsy of this animal revealed that the extirpation of the stomach on this animal was complete.

We are indebted to the kindness of Dr. E. L. Opie for the following report on the autopsy: — The mucous membrane is 2 mm. in thickness, the villi appear to be considerably larger than normal and the glands longer; otherwise, the tissue appears to be normal.

SUMMARY.

1. Observation on the fate of the nitrogen intake received in addition to that of the standard diet was studied on two animals with gastrectomy.

2. In the first animal there was noted a high nitrogen retention in the experiments performed early after the operation, and no retention in the experiments performed after the tenth week following the operation.

3. The observations on the second animal were begun on the twelfth week after the operation. Three experiments were performed on the animal and no nitrogen retention noted in any one of them.

4. The autopsy revealed that in the first animal a small part of the pyloric end, not more than 15 mm. in length, remained unexcised. The extirpation of the stomach in the second animal was complete.

5. Parenterally introduced protein (in addition to the standard diet) was completely retained in the organism.

6. An analysis was made of the possible conditions which might have occasioned the divergence in the behavior of the additional nitrogen in the experiment following early after the operation and those after a lapse of about ten weeks.

7. The view was analyzed that immediately after the operation not only the gastric secretion was missing, but also the pancreatic and in-

testinal secretions were minimal, and that at that period the absorbed protein had the character of parenterally introduced protein; and that at a later period the pancreatic and intestinal digestion were restored to their normal power.

8. The autopsy revealed a hypertrophy of the upper end of the duodenum developing after the operation.

THE PROPHYLACTIC ACTION OF ATROPIN IN IMMEDIATE ANAPHYLAXIS OF GUINEA PIGS. — THIRD COMMUNICATION.

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INTRODUCTION.

ABOUT one year ago it was stated in a preliminary note [1] that atropin sulphate could prevent the death of a guinea pig from a very acute type of anaphylaxis which we later termed the immediate anaphylactic reaction.[2] We first used atropin because the experimental facts which we obtained in our investigation all pointed to the conclusion that the bronchial muscles, especially those of the finer tubes, played an important, if not the most important, rôle in the production of the pulmonary stenosis which led swiftly up to the death of the animal by asphyxia.[3] On the basis of this conclusion, which has since been adopted by Anderson and Schultz [4] and by Biedl and Kraus,[5] it was natural that atropin should be employed because of its well-known paralyzing action upon the vagus endings in the bronchial muscles. This action of atropin was first shown by Dreser [6] and was later corroborated by Beer,[7] Einthoven,[8] and by Dixon and Brodie.[9] The thera-

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[1] AUER and LEWIS: Journal of the American Medical Association, 1909, viii, p. 458.

[2] AUER and LEWIS: Journal of experimental medicine, 1910, xii, p. 153.

[3] AUER and LEWIS: *Ibid.*, pp. 165–169.

[4] ANDERSON and SCHULTZ: Proceedings of the Society for Experimental Biology and Medicine, 1909, vii, p. 34.

[5] BIEDL and KRAUS: Wiener klinische Wochenschrift, 1910, xxiii, No. 11, p. 386.

[6] DRESER: Archiv für experimentelle Pathologie und Pharmakologie, 1890, xxvi, p. 255.

[7] BEER, Archiv für Physiologie, 1892, Supplement-Band, p. 150.

[8] EINTHOVEN: Archiv für die gesammte Physiologie, 1892, li, p. 428.

[9] DIXON and BRODIE: Journal of physiology, 1903, xxix, pp. 162, 168.

peutic results we obtained with this alkaloid in an experimental test were gratifying and were reported in our preliminary note. Since that time some series of experiments have been carried out in guinea pigs of varying sensitiveness, and these results will be briefly reported in the following pages.

<div align="center">EXPERIMENTS.</div>

In order that a clear conception of the course of these experiments may be obtained, two specimen protocols will be given which may serve as types, and the experiments, which will be presented later in tabular form, were carried out in this fashion.

Control Experiment, June 30, 1910. — G. pig, male, F 16; 700 gm. Sensitized Nov. 17, 1909, by 1 c.c. H. S. subc.

10.27.   Stretched on electric pad at L.   Start ether.

10.29.   Cannula in external jugular vein; stop ether.

10.31.   Rectal temperature 38.8°.

10.35.   0.5 c.c. 10 per cent heated H. S. jug. vein; 1½ c.c. Ringer sol.

10.36.   Chest sinks in fairly well with inspiration.   Respiration slowed.

10.36½.   Chest sinks in strongly with each inspiration.   Respiration slow; struggles, no sound audible.

10.37.   Chest sinks in strongly with inspiration; tongue bluish; struggles, choked squeak.

10.39.   Short convulsions; no respiratory sound audible.

10.40.   Mouth opens with inspiration; chest movements getting less and less; visible peristalsis.

10.41.   No respiration. Rectal temperature 38.7°.

Autopsy: Lungs typical, large, full, pale bluish pink; left upper lobe only slightly distended; no difference between two sides otherwise.

Atropin. June 30, 1910. — G. pig, male, F 19; 700 gm. Sensitized Nov. 17, 1909, by 1 c.c. H. S. subc.

11.40.   Stretched on electric pad at L.   Start ether.

11.44.   Cannula in external jugular vein; stop ether.

11.50.   Rectal temperature 39.9°.

11.51.   2 mg. atropin sulph. jug. vein (1 per cent solution). 0.5 c.c. 10 per cent heated H. S. jug. vein; 1½ c.c. Ringer sol.

11.52½.   Slight sinking in of chest with inspiration.

11.53.   Sharp struggle with some rather choked squeaks.

11.55.   Slight sinking in only of chest; sharp struggles with choked squeaks.

11.56. Same; during struggles air heard issuing from mouth and nose.

11.57. Same; only slight sinking in of chest; respiration moderately rapid.

11.59. Chest sinks in a bit more. 1 mg. atropin, jugular vein; 1 c.c. Ringer sol. to wash out cannula.

12.05. Chest sinks in only very moderately with inspiration. Wound washed with 2.5 per cent carbolic sol. Sutured; squeaks slightly as needle passes through skin. Rectal temperature 40.4°. Placed in box.

12.08. Moves about box; holds head up; hair of head and neck erect, smooth over rest of body. Respiration rapid. Occasionally hind legs straighten abruptly and raise rump of animal.

1.20. Good condition.

3.40. Good condition.

July 1, 10 A. M. Lively and active.

July 7. Well, lively and active.

The protocols quoted above show well that the lungs are markedly involved in immediate anaphylaxis and that atropin reduces these manifestations strongly. Yet the significance of the protocols is not, perhaps, obvious, and for this reason a graphic record of the lung changes themselves is given. Fig. 1 shows these changes very well. This tracing was obtained from a guinea pig which had been sensitized June 4, 1909, by the subcutaneous injection of 5 c.c. of meningitis serum. On September 27, 1909, this animal was stretched out on an electric pad, etherized, both vagi cut; a pleural cannula fixed in the right pleural cavity; 2.5 mg. curarin injected into the external jugular vein and artificial respiration started. The volume changes of the lung were recorded by connecting the pleural cannula with a Marey tambour, upstroke of the writing lever being caused by inflation of the lung. Both vagi were first stimulated to cause a bronchial tonus (not reproduced in tracing). Then 0.6 c.c. of anti-meningitis (horse) serum was injected into the jugular vein and the cannula washed clear by 1 c.c. of saline solution. Within a few seconds (time is marked in four-second intervals on tracing) and before the salt solution is injected, a well-marked increase in the lung volume oscillations occurs, a broncho-dilatation,[10] which is followed swiftly by a broncho-constric-

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[10] AUER and LEWIS: Journal of experimental medicine, 1910, xii, pp. 167, 168. This has also been observed by BIEDL and KRAUS: *Loc. cit.*, p. 386.

tion of such a degree that the air blasts from the artificial respiration machine hardly cause any volume changes in the lung, although the volume, force, and rate of the air delivery has in no wise been altered. The animal now receives practically no air, and if it were not curarized would show powerful struggles followed swiftly by asphyctic convulsions and death.[11]   In the tracing under consideration, however, con-
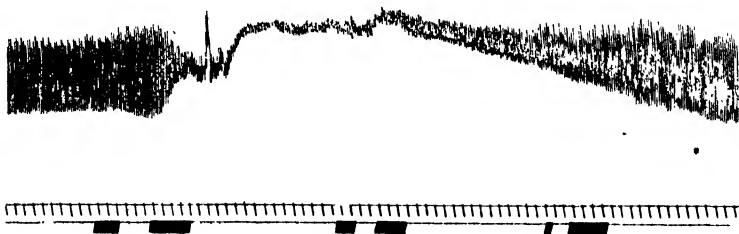
FIGURE 1. — From the right pleural cavity of a sensitized curarized guinea pig September 27, 1909.   Upstroke = inflation of lung under artificial respiration from a machine. Time = four-second intervals.   At once after the intravenous injection of 0.6 c.c. of horse serum (first black band), a short but well-marked broncho-dilatation occurs. This is swiftly followed by an almost maximal broncho constriction.   During this broncho-constriction 3 mg. atropin and again 2 mg. were injected into the jugular vein. Note prompt return of lung oscillations.

ditions were not allowed to progress to this pass, but 3 mg. of atropin were injected into the jugular vein.   Very swiftly, a matter of seconds, the volume changes of the lung, due to the artificial respiration, begin to increase, showing that the pulmonary stenosis, which had been produced by the horse serum, was slowly yielding to the atropin.   A second injection of atropin, this time 2 mg., was given, perhaps unnecessarily, to hasten the removal of the stenosis, and two and one-half minutes after the first atropin injection the lung again expands and collapses to artificial respiration even better than before the injection of the toxic dose.   This tracing (Fig. 1) shows at a glance the kernel of this communication.

While this tracing shows well the vital functional interference which horse serum causes in a sensitized guinea pig and its removal by atropin, there is nothing which conveys to the eye the marked gross anatomical change which accompanies this alteration of lung function.[12]   In order to show this and also the effect of atropin, two guinea pigs from the

[11] See Plate VIII, Auer and Lewis: *Loc. cit.*, for a graphic registration of this.
[12] Auer and Lewis: *Loc. cit.*, p. 157.

same lot, sensitized by the subcutaneous injection of 1 c.c. of horse serum on November 17, 1909, received on January 24, 1910, intravenously 0.3 c.c. of a 10 per cent solution of heated horse serum.[13] One of these guinea pigs had received 3 mg. of atropin subcutaneously about fourteen minutes before the toxic dose of horse serum. The control animal died with typical respiratory symptoms in five minutes, while the atropin animal showed practically no respiratory involvement during ten minutes of observation, when it was killed by section of the medulla. The lungs of these animals were excised and photographed. Fig. 2 is a reproduction of this photograph. Inspection of this picture shows that the difference between the two lungs is striking: The control lungs (*b* in the picture) are pale, full, and light of weight; they seem to be fixed in an inspiratory condition in spite of the fact that they are excised from the thorax and that there is no mechanical obstruction in the trachea; moderate tracheal inflation would exert no effect on the lung volume.[14] They are full of air which cannot escape, and inspection of the lung surface with the naked eye shows beautifully the distended alveolar air-sacs. If the lung surface had been pricked by a needle (which was done in numerous other experiments), air mixed with a little blood would have been seen bubbling out as if under some tension. In short, the lungs of this control animal look exactly like normal lungs at the end of a full inspiration;[15] the only difference is that these anaphylactic lungs maintain this inspiratory condition when removed from the thorax, the atmospheric pressure and the pull of the stretched elastic tissue, which strain to empty the lung, being counteracted by a stenosis of the pulmonary air passages.[16] These lungs again indicate clearly why the animal died of asphyxia. On the other hand, the lungs of the atropin animal (*a* in Fig. 2) show a very different appearance: they are collapsed, small, and almost air-less, about one half the size of the anaphylactic lungs; their color is a dark gray, and the surface of the lungs shows numerous

[13] It must be added that in both animals the right vagus had been resected on January 11. I have shown in another place (Proceedings of the Society of Experimental Biology and Medicine, 1910, vii, p. 104) that this has no effect on immediate anaphylaxis.

[14] AUER and LEWIS: *Loc. cit.*, p. 162; also BIEDL and KRAUS: *Loc. cit.*, p. 386.

[15] For a fuller description of the typical anaphylactic lung see AUER and LEWIS: *Loc. cit.*, p. 156.

[16] The production of this stenosis is shown in Fig. 1.

fine wrinkles (not well brought out in the photograph); on immediate moderate rhythmic inflation these lungs would expand and collapse readily; in short, these atropin lungs look like those which may be seen in any normal guinea pig shortly after death when the thorax is opened. There is one exception, however, to be noted; the right middle lobe looks pale and distended, and this lobe resembles the lungs of the control animal; this lobe is fixed in a more or less inspiratory condition and contains a good amount of air; the significance of this partial inspiratory immobilization will be considered in another place. From this description it may readily be seen that the impairment of lung function which horse serum produced in the sensitized guinea pig had been successfully decreased by atropin so that it no longer menaced the life of the animal; here atropin exerted a curative effect.

FIGURE 2. — Lungs from two guinea pigs of the same lot. Magnification about 2 X. Both guinea pigs were sensitized November 19, 1909, by the subcutaneous injection of 1 c.c. horse serum. On January 11, 1910, the right vagus was resected in the neck of each. On January 24, 1910, the animal, whose lungs are marked *a* in the photograph, received 3 mg. of atropin subcutaneously; fourteen minutes later the toxic dose, 0.3 c.c. of a 10 per cent solution of heated horse serum, was injected into the jugular vein; ten minutes later the animal was killed by section of the medulla. The lungs were then excised and photographed together with those of the control animal. The control (marked *b* in photograph) received the same dosage of horse serum, but no atropin. It died with typical respiratory symptoms within five minutes after the injection.

In the foregoing pages proof of various kinds has been submitted that atropin may prevent or abolish the death-producing inspiratory immobilization of the guinea pig's lungs in immediate anaphylaxis. There remains to be shown that this life-saving action of atropin occurs in a good percentage of the cases where this drug is used, and for this purpose a few tables will be presented.

ATROPIN STATISTICS.

All the guinea pigs whose records will form one table belong to the same lot and are practically of the same age and weight. This holds

true also of Table I, where the guinea pigs were sensitized at two different times and with different amounts of serum. The course of each experiment is shown by the two protocols quoted on preceding pages. Atropin was always used in the form of a sulphate and in a 1 per cent solution; when injected subcutaneously, it was given in the

TABLE I.

Animals sensitized Fourteen to Sixteen Days. Average Weight, 400 Gm.

No.	Sensitized.	Date of toxic dose.	Atropin.	Toxic dose.	Result.
E 5	Nov. 17, '09. 1 cc. H.S. subc.	Dec. 3, '09. 16 days	3 mg. subc.	0.6 cc. heated H.S. jug. vein.	Slight symptoms. Recovery.
E 4	" "	" "	None.	" " "	Death in 5 min.
E 6	" "	" "	3 mg. subc.	" " "	Slight symptoms. Recovery.
E 102	March 14 1 c.c. 10% H.S. subc.	March 28 14 days	None.	0.4 c.c. heated. H.S. jug. vein	Death in 4 min.
E 103	" "	" "	3 mg. subc. 1½ mg. jug. vein.	" " "	Death in 6 min.
E 104	" "	" "	None.	0 3 c.c. heated H.S. jug. vein.	Death in 9 min.
E 105	" "	" "	5 mg. subc. 1½ mg. jug. vein.	" " "	Death in 75 min.
E 107	" "	" "	None.	" " "	Slight symptoms. Recovery.
E 108	" "	" "	5 mg. subc. 1 mg. jug.	0.4 c.c. heated H.S. jug. vein	Slight symptoms. Recovery.
E 110	" "	" "	None.	" " "	Death in 4 min.
E 109	" "	" "	4 mg. Subc. 3 mg. jug.	" " "	Good chest symptoms. Recovery.

6 atropin pigs : 2 died = Death 33% = Recovery 66%.
5 controls   : 4 died = Death 80% = Recovery 20%.

right upper abdominal quadrant and eleven to fifteen minutes allowed to elapse before the injection of the toxic dose of serum. All intravenous injections were made into a cannula ligated in the external jugular vein, the injection of atropin or serum being followed by 1 c.c. of saline or Ringer solution to wash out the cannula. The horse serum employed was almost always heated to 55° for thirty-five minutes to reduce its toxicity, and the dose was about the minimal lethal dose. The slight operation required for this work was done under primary ether anæsthesia as a rule, and about five minutes were allowed to

TABLE II.

ANIMALS SENSITIZED TWENTY-ONE TO TWENTY-SEVEN DAYS. AVERAGE WEIGHT, 300 GM.

No.	Sensitized.	Date of tox. dose.	Atropin.	Toxic dose.	Result.
D 131	Oct. 1, 1909 1.c.c. H.S. subc.	Oct. 22, 21 days		0.5 c.c. heated H.S jug. vein	Death in 5 min.
D 132	" "	" "	1 mg. subc.	" "	No chest symptoms. Recovery.
D 133	" "	" "		" "	Mod. chest symptoms. Recovery.
D 134	" "	" "	1 mg. subc.	" "	Death in 4 min..
D 135	" "	24 days		" "	Death in 4 min.
D 136	" "	" "	3 mg. subc.	" "	Slight symptoms. Recovery.
D 138	" "	" "		" "	Moderate symptoms. Died during night.
D 137	" "	" "	3 mg. subc.	" "	Slight respiratory symptoms. Recovery
D 139	" "	" "		" "	Death in 4 min.
D 140	" "	" "	3 mg. subc.	" "	No symptoms. Recovery. Lively next day.
D 141	" "	25 days		" "	Good respiratory symptoms. Recovery.
D 143	" "	" "	3 mg. subc.	" "	No symptoms (resp.) during 11 min. Killed to study lungs.
D 142	" "	" "		" "	Died in 4 min.
D 144	" "	" "	3 mg. subc.	" "	Slight respiratory symptoms after 14 min. Killed to study lung.
D 145	" "	" "		" "	Death in 5 min.
D 146	" "	26 days		" "	Death in 4 min.
D 148	" "	" "	3 mg. subc.	" "	Death in 22 min. Very sick, but only mod. resp. symptoms.
D 147	" "	" "		" "	Death in 6 min.
D 149	" "	" "	3 mg. subc.	" "	Very slight respiratory symptoms. Recovery.
D 150	" "	27 days		0.4 c.c. heated jug. vein	Death in 5 min.
D 151	" "	" "	3 mg. subc.	" "	Very slight resp'y symptoms after 9 min. Killed to study lungs.
D 154	" "	" "		0.5 c.c. heated H.S. j. v.	Death in 5 min.
D 155	" "	" "	3 mg. subc.	" "	No respiratory symptoms during 15 min. Killed to study lungs.

11 atropin pigs : 2 died = Death 18% = Recovery 82%.
12 controls   : 9 died = Death 75% = Recovery 25%.

pass after stoppage of the ether to permit full recovery from the effects of the anæsthetic. The loss of heat, which occurs swiftly in such a small animal as the guinea pig when it is stretched out at full length,

TABLE III.

ANIMALS SENSITIZED TWO HUNDRED AND TWENTY-FOUR TO TWO HUNDRED AND TWENTY-SIX DAYS. AVERAGE WEIGHT, 700 GM.

No.	Sensitized.	Date of tox. dose.	Atropin.	Toxic dose.	Results.
	Nov. 17, 1909.	June 29, '10			
F 11	1 c.c. H.S. subc.	224 days	....	0.3 c.c. 10% h'd H.S. j. v.	Death in 7 min.
F 13	" "	" "	3 mg. subc. 1 mg. j. v.	" "	Slight respiratory symptoms. Recovery.
F 14	" "	" "	....	" "	Moderate respiratory symptoms.
F 12	" "	" "	3 mg. subc. 2 mg. j. v. 1 mg. j. v.	" "	Death in 3 min.
F 16	" "	225 days	....	0.5 c.c. 10% h'd H.S. j. v.	Death in 6 min.
F 19	" "	" "	3 mg. j. v. 1 mg. j. v.	" "	Slight resp. symptoms. Recovery.
F 21	" "	" "	....	" "	Death in 13 min.
F 17	" "	" "	3 mg. subc. 4 mg. j. v. (4 doses.)	" "	Death in 14 min.
F 24	" "	" "	....	" "	Moderate resp. symptoms. Recovery.
F 22	" "	" "	2 mg. j. v. 1 mg. j. v.	" "	Death in 2 min.
F 23	" "	" "	2 mg. j. v.	" "	No resp. symptoms. Lively next day.
F 26	" "	226 days	....	" "	Death in 3 min.
F 27	" "	" "	2 mg. j. v.	" "	Very slight symptoms. Recovery. Lively next day.
F 30	" "	" "	....	" "	Death in 4 min.
F 28	" "	" "	2 mg. j. v. 1 mg. j. v.	" "	Mod. resp. symptoms. Recovery.

8 atropin pigs : 3 dead = Death 38% = Recoveries 62%.
7 controls   : 5 dead = Death 71% = Recoveries 29%.

was prevented by placing the animal on an electric heating pad. It will be noted that for practically every atropin experiment there is a control experiment.

Whenever "recovery" is noted in the protocols it means that the animal was observed at least two days after the experiment.

Four atropin animals which showed practically no respiratory symptoms were killed after nine to fourteen minutes in order to study their lungs. As the controls usually died within five minutes showing marked respiratory involvement, these four animals are classed as recoveries.

DISCUSSION.

From the preceding tables it will be seen that atropin exerts a clear-cut prophylactic effect in a very acute type of serum anaphylaxis which Lewis and I have termed immediate anaphylaxis. Out of twenty-five guinea pigs which had been sensitized from fourteen to two hundred and twenty-six days, atropin saved eighteen, or 72 per cent, while out of twenty-four guinea pigs which served as controls for the atropin series, only six survived, or 25 per cent. Stated otherwise, the death rate in the atropin series was 38 per cent, while in the control series it was 75 per cent. These results substantiate well the statement made in our preliminary report.[17]

Since this preliminary report first appeared, the effect of atropin in serum anaphylaxis was tested by Anderson and Schultz[18] and by Biedl and Kraus.[19] Anderson and Schultz succeeded in saving only about 28 per cent of their animals (4 out of 14) by the use of atropin. These observers used young guinea pigs weighing about 300 gm. after they had been sensitized by an intra-orbital injection of 0.01 c.c. of horse serum for twenty-one to thirty days. The toxic dose was 0.5 c.c. of the same serum injected intravenously. Atropin was given "usually intraperitoneally" in 3 mg. doses. They do not state how much time was allowed to elapse between the injection of the atropin and the administration of the toxic dose of serum. This latter point is of importance because the effect of atropin on the lungs is by no means permanent. There is a certain optimum time for the injection of the toxic dose after atropin has been given, and we may with justice assume that this is apparently when the balance between the absorption and excretion of the drug is such that its concentration in the blood is

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[17] AUER and LEWIS: Journal of the American Medical Association, 1909, viii, p. 458.

[18] ANDERSON and SCHULTZ: Proceedings of the Society for Experimental Biology and Medicine, 1909, vii, p. 35.

[19] BIEDL and KRAUS: Wiener klinische Wochenschrift, 1910, xxiii, p. 387.

greatest. This time naturally will vary with the mode of administration of the alkaloid. As I have no experience of my own with the prophylactic effect of atropin when given intraperitoneally, it would be useless for me to speculate on the causes for the meagre yield of therapeutic results in the hands of Anderson and Schultz.

The other observers who used atropin were Biedl and Kraus. They injected the drug intravenously in guinea pigs, the doses varying from 1 to 10 mg., and were successful in abolishing the anaphylactic immobility of the lung. They also found that 5 mg. given prophylactically prevented the appearance of lung symptoms, but these investigators apparently have not tested what percentage of success they could obtain in the attempt to save sensitized guinea pigs from the fatal effect of serum when injected the second time.

Mode of administration of atropin.— In earlier work atropin was always given subcutaneously some ten minutes before the toxic dose of serum. This mode of incorporating the drug has the drawback that there is no definite knowledge when the lung is most under the influence of the alkaloid, for it is impossible to tell in the ordinary experimental test when the ratio between absorption of the alkaloid and its excretion has reached that point where the blood contains most of the substance. This problem is still further complicated by the fact that the "subcutaneous" injections are often partially intradermal or intramuscular, combinations which vitally affect the rate of absorption. Now, since the rôle of atropin and toxic dose of serum is that of antagonists, at least as far as the bronchial muscles are concerned,[20] it is obvious that the most favorable time for injection of the toxic dose is when the blood contains most of the atropin. For this reason I have lately used the intravenous route for the injection of the prophylactic dose of atropin. As a rule 2 mg. of the drug in a 1 per cent solution is injected into the external jugular vein and the cannula washed out by 1 c.c. of Ringer solution. Now after the lapse of ten to fifteen seconds the toxic dose of horse serum is injected also intravenously. Done in this fashion, the experimenter is certain that the maximum amount of the atropin swiftly comes within striking dis-

[20] I have demonstrated recently that the bronchial muscles themselves are sensitized (Proceedings of the Society for Experimental Biology and Medicine, 1910, vii, p. 104), and that atropin paralyzes the bronchial muscles and not only the nerve endings (Journal of experimental medicine, 1910, xii, No. 5).

tance of the bronchial muscles, and moreover he is fairly sure of the time when this occurs. Another advantage of this method is that further injections of atropin may swiftly be sent to the bronchial muscles, if the animal shows the characteristic chest signs that the toxic dose of serum is gaining the upper hand. Care must, however, be exercised not to send in too much atropin, for an excess of this drug will paralyze the respiratory centre. I feel certain that in a number of cases I gave too much atropin, and thus aided in killing the animal when I was attempting to save it. The drug also should not be injected too swiftly nor in too concentrated a form.

Toxic dose. — In order to demonstrate the prophylactic effect of atropin in immediate anaphylaxis, I have always attempted to use the minimal lethal dose of horse serum for the second or toxic injection. This was done so that the effect of atropin could be observed most clearly with the smallest dose of the drug. I have made no attempt so far to establish how many lethal doses of horse serum a sensitized guinea pig will stand with the aid of atropin. During the course of these experiments it was soon found that the minimal lethal dose of the serum, which was established practically every time that a batch of experiments was carried out, showed at first a shifting in value with increasing length of sensitization. For example, some guinea pigs, sensitized on November 17, 1909, by the subcutaneous injection of 1 c.c. of horse serum, were tested on December 3 (sixteen days), when 0.6 c.c. heated horse serum injected into the jugular vein killed within five minutes; 0.4 c.c. of the same serum gave only slight respiratory symptoms in another animal. On January 24, 1910 (sixty-eight days), another test was made: 0.2, 0.1, 0.04 c.c. heated horse serum, all killed the guinea pigs within five minutes when injected intravenously; 0.02 c.c. caused powerful respiratory symptoms from which the animal recovered. On June 30, 1910, two hundred and twenty-five days after sensitization, it was found that the sensitiveness of the animal was practically the same as after sixty-eight days: 0.05 c.c. usually killed within five minutes. From these data it will be seen that the sensitiveness of the injected guinea pigs increased until the maximum was reached on or before sixty-eight days, when one fifteenth of that dose killed which was necessary sixteen days after sensitization; and this level was found maintained two hundred and twenty-five days after sensitization. These facts support and supplement respectively some

statements by Lewis [21] and by Rosenau and Anderson.[22] Lewis found that the maximum of hypersensitiveness, when tested by the subcutaneous method of introducing the toxic dose, was reached in about three or four weeks. Unfortunately I made no tests between the sixteenth and the sixty-eighth day, and it is very well possible that this maximum occurs well ahead of the latter time. Rosenau and Anderson tested animals after intervals of more than seven hundred and one thousand days and found the animals still extremely sensitive. These authors, however, injected about 6 c.c. of the horse serum intraperitoneally for the toxic dose and their results have therefore only a qualitative value. The results I described briefly above show that there is practically no quantitative fluctuation in the amount of the killing dose of horse serum for at least twenty-three weeks after the maximum sensitization level has been reached.

Action of atropin in immediate anaphylaxis. — This phase of the problem need not be considered here in connection with the present study, for it has been reported in another place.[23] It will be sufficient to state that proof has been advanced that in anaphylaxis the bronchial muscles themselves are sensitized by horse serum and that atropin in proper dosage is able to paralyze the denervated bronchial musculature. I was unable to find any evidence for any rôle which the vagus broncho-motor endings might play in immediate anaphylaxis.

SUMMARY.

A prophylactic injection of atropin sulphate in guinea pigs sensitized by the subcutaneous injection of horse serum saved eighteen out of twenty-five from the lethal effect of the toxic injection; while out of twenty-four adequate controls only six survived. Stated otherwise: The death-rate with atropin was 28 per cent; without atropin it was 75 per cent. These figures show the distinct therapeutic utility of atropin in immediate anaphylaxis.

[21] LEWIS: Journal of experimental medicine, 1908, x, p. 6.

[22] ROSENAU and ANDERSON: Hygienic laboratory bulletin, No. 45, 1908, p. 57, and No. 50, 1909, p. 47.

[23] AUER: Proceedings of the Society for Experimental Biology and Medicine, 1910, vii, p. 104; AUER: second communication, Journal of experimental medicine, 1910, xii, No. 5.

Guinea pigs sensitized by the subcutaneous injection of one or two cubic centimetres of horse serum, reach their maximum sensitiveness on or before the ninth week, and this sensitiveness, tested by intravenous injection of the toxic dose, is then practically *quantitatively* maintained for at least twenty-three weeks (the longest interval tested).

EXPERIMENTAL YAWS IN THE MONKEY AND RABBIT

By HENRY J. NICHOLS

EXPERIMENTAL YAWS IN THE MONKEY AND RABBIT.[1]

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PLATES LIII AND LIV.

INTRODUCTION.

Yaws or frambesia has always been of particular interest on account of its possible relation to syphilis, and since the discovery of its cause it has been studied with renewed activity. The discovery of *Treponema pertenui,* by Castellani (1) in 1905, was a direct sequel to Schaudinn's work on syphilis, and both Schaudinn and Castellani considered the organisms of the two diseases morphologically identical. More recently, minute differences in form have been pointed out by Prowazek (2) and Russell (3); but whether or not they are to be accepted as sufficient for differentiation, there is no doubt, on the basis of immunity experiments on monkeys (4) and man (5), that the two diseases are distinct. In Castellani's words, "Yaws is not syphilis any more than leprosy is tuberculosis."

In the recent study of syphilis, a great advance has been made by Parodi (6) and Uhlenhuth and Mulzer (7), who have demonstrated the possibility of producing a syphiloma in the testicle of the rabbit. As Uhlenhuth and Mulzer say, this lesion gives us a convenient and rich source of spirochetæ, in pure culture, with which cultivation and immunity experiments can be carried out.

Several months ago, I undertook some work on syphilis in the rabbit's testicle and, as a case of yaws was under my observation

at the time, I thought it worth while to attempt to infect rabbits with the spirochetæ of yaws as well as with those of syphilis. This attempt has been successful and I have been able to observe a parallel series of infections.

The spirochetæ of yaws came from a colored soldier returning from the Philippines (8). This soldier, forty years of age, had a well-marked case of yaws consisting of three separate general eruptions and marked joint symptoms. Spirochetæ in small numbers were found in the serum from the yaws on two occasions with the dark field microscope (Plate LIII, Fig. 1).

EXPERIMENTS.

On Dec. 17, 1909, a monkey (*Macacus rhesus* 1) was inoculated by rubbing an excised piece of a yaw into an abraded surface of the right eyebrow. The abrasion healed completely in a few days. On Jan. 7, 1910, 24 days after inoculation, a red and slightly scaly patch was noticed on the right eyebrow, and in a few days a well-marked red and edematous ridge of tissue developed. Spirochetæ in small numbers were repeatedly found in fluid expressed from this lesion. The growth invaded the upper eyelid, ulcerated, and on Feb. 2 showed a typical yellow crust formed by the rich serous discharge from fungoid granulations (Plate LIV, Fig. 2).

Three rabbits were inoculated in the testicle with serum from the lesion in the monkey. All became infected as follows: Jan. 27, the unbroken surface of the edge of the monkey's yaw was slightly abraded, a drop of clear serum was expressed, taken up in a capillary pipette and expelled by air pressure into the substance of the right testicle of a large white rabbit, A. Weekly examinations were made and no change was noticed until Feb. 24, 28 days after inoculation, when the right testicle was found uniformly increased in size so that it could not be retracted; it was firm throughout but more resistant at the upper part. On Feb. 25, the resistant area was punctured with a sterile capillary pipette and a drop of clear gray fluid obtained which, under the dark field microscope, showed a great number of long active spirochetæ of the *pertenuis* or *pallida* type. The second rabbit, A¹, inoculated in the same way, showed an enlarged testicle containing a small round nodule rich in spirochetæ after 43 days. The third rabbit, C, became infected after 52 days.

With material from the first rabbit, A, the disease was *reproduced in the monkey and continued in a second generation in rabbits.*

March 9, a drop of serum from the testicle of Rabbit A, rich in spiro chetæ, was rubbed into the eyebrow of a monkey (*Macacus rhesus* 2). In 16 days a red papular area appeared and soon became markedly elevated, edematous and slightly scaly. Spirochetæ were readily found in the expressed fluid.

Feb. 25, 8 rabbits were inoculated with fluid from the testicle of Rabbit A; 3 with undiluted fluid and 5 with fluid diluted with 3 parts of salt solution. One of the 5 died after 14 days of rabbit septicemia, and there was no change

in the testicle and no spirochetæ were found. Two of the rabbits inoculated with undiluted fluid developed lesions rich in organisms 32 and 42 days after inoculation. One of the 4 remaining showed an infection after 52 days. All these rabbits were undersized and gray or brown.

For the third generation larger animals were selected. March 29, 2 large white rabbits were inoculated in the right testicle by syringe with about .5 c.c. of a mixture of about 3 parts of salt solution and one part of fluid from a nodule in the testicle of Rabbit 7 (second generation). April 18, after 20 days, one showed an enlarged testicle with a distinct nodule very rich in active spirochetæ. April 22, after 24 days, the other showed the same. March 31, Rabbit 7, of the second generation, was castrated under ether anesthesia; a white edematous tumor was found occupying the middle of the testicle. Small pieces of this tumor were introduced in the testicles of 4 large brown rabbits with a trochar and canula; on April 22, after 22 days, Rabbit 14 showed a definite nodule rich in spirochetæ. Two other animals, Nos. 15 and 17, showed lesions after 36 and 22 days.

Thus the spirochetæ of yaws have been transferred from man to the monkey and from the monkey through three generations in rabbits. Eight out of sixteen rabbits used in this series have become infected. The incubation period averaged forty-one days in the first generation, forty and a half days in the second generation and twenty-four and eight-tenths days in the third generation, thus showing a shortening of sixteen days in the third passage. The series is being continued and, by using care in the selection of animals, can probably be continued indefinitely. Large animals are more suitable than small and in case of both syphilis and yaws the order of susceptibility seems to be white, gray, brown and black. I do not know yet which is the best medium for transfer, undiluted serum, salt solution, citrate solution or the bits of tissue: all have been successful. Inoculation in the anterior chamber of the eye has as yet failed of results. In tapping the testicle, if the point of a moderately strong capillary pipette is guided into the nodule, spirochetæ, in pure culture, can be obtained in myriads; in the testicle away from the nodule they are found in small numbers. They are readily stained by Giemsa's method (Plate LIV, Fig. 3). The dark field microscope is almost indispensable in handling a large number of animals. Swelling of the testicle and edema of the tunica vaginalis may result from frequent tappings, but no mixed infections of the testicles have occurred. The general health of the rabbits does not seem to suffer.
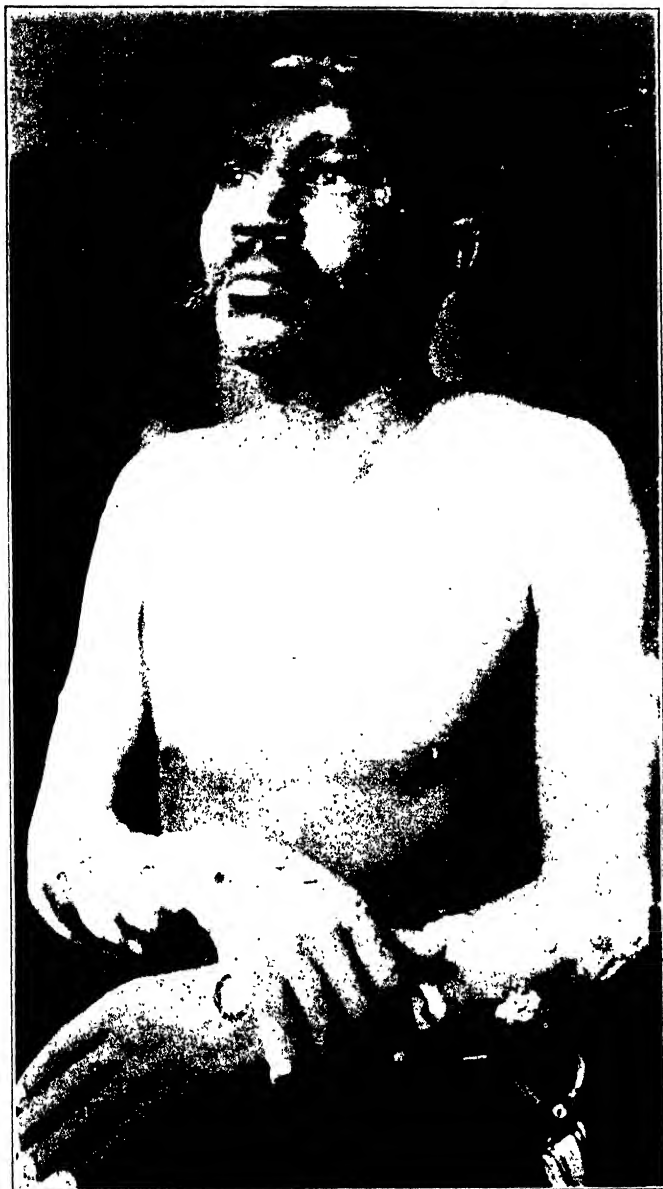
FIG. 1.

FIG. 2.

FIG. 3.
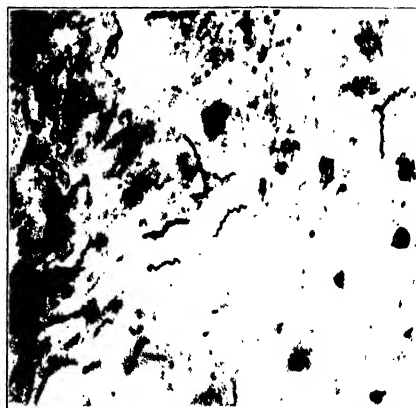
FIG. 4.

The Lesion of Yaws in the Rabbit's Testicle.—Three rabbits were castrated, under ether, thirty-four, forty-eight and sixty days after inoculation and the tissue used for histological study. If the testicle is examined soon after a nodule is palpable, the nodule is found to be a whitish edematous tumor fairly well differentiated from the rest of the testicle which appears normal. On incision, the edges of the growth roll back and a good deal of serum escapes. The tissue appears homogeneous. Sections show a picture of an interstitial orchitis; the tubules are small, disintegrating or replaced by an edematous connective tissue containing an infiltration of small round and endothelial cells. New polymorphonuclear cells are seen. In sections stained by Levaditi's method spirochetæ are found in large numbers in and around the tubules and in the interstices of the connective tissue (Plate LIV, Fig. 4). In the testicles examined after forty-eight days the infiltrating cells had for the most part disappeared and after sixty days the connective tissue was more fully organized and no spirochetæ were found. Repeated tapping of these testicles may have somewhat changed the natural history of the lesions but apparently the lesion resolves in time, leaving an atrophied organ.

Serum Reactions.—The complement fixation reaction has been tried on the yaws rabbits and has been positive in several cases.

.1 c.c. of serum heated at 56° C. for 15 minutes, acetone insoluble liver extract as antigen, and the human corpuscle and rabbit serum hemolytic system were used.

White Rabbit No. 6, second generation. Feb. 25, inoculated with undiluted serum from Rabbit A. March 1, serum negative; April 8, nodule palpable (42 days), spirochetæ present; April 9, serum negative; April 19, serum positive (53 days).

In the second case a positive reaction led to a reëxamination and the finding of spirochetæ.

Gray Rabbit No. 3. Feb. 25, inoculated with diluted serum from Rabbit A. Serum negative; April 18, testicle increased in size; no definite nodule (52 days); April 19, spirochetæ not found in one slide with dark field microscope; April 20, serum taken on April 19, positive, April 21, spirochetæ found (55 days).

The patient's blood was negative on four examinations, November 20, December 14, February 23 and March 15, after the second and third eruptions and after treatment with potassium iodide. Both monkeys have failed as yet to give the reaction. There are

two examples of the reaction in yaws on record, in both of which it was positive. Bruck (9) gives the case of a thirty-year-old Malayan who had yaws five years before. Hoffman (10) records a case of a native of Suaheliland with a positive reaction at the time of the eruption. The diagnosis was doubted by Ziemann. If, as seems probable, this reaction is not a specific immunity reaction, but rather a result of the breaking down of certain kinds of tissue, a good deal may possibly be learned from the study of the reaction in closed lesions in such a specialized organ as the testicle.

Syphilis in the Rabbit's Testicle.—A comparison of yaws with syphilis in the rabbit brings out several points of importance. January 27, a patient with mucous patches of the mouth and anus, untreated for three months, and with a clear history of a primary sore and a secondary eruption, was secured through the kindness of Dr. Seay. A patch on the lower lip was cleaned, aspirated with a suction apparatus and a few drops of serum containing numerous spirochetæ was injected with a capillary pipette into the left testicle of five large rabbits.

Feb. 28, testicle of Rabbit E enlarged; cannot be retracted; nodule felt; spirochetæ numerous (32 days).

Two other rabbits showed infection after 35 and 56 days.

Jan. 31, 5 rabbits inoculated from mucous patch; 2 were infected after 45 and 64 days. One of these rabbits is especially interesting as the infection travelled over to the uninoculated testicle and the Wassermann reaction appeared, disappeared and reappeared.

Jan. 31, large gray rabbit, G, inoculated right testicle from mucous patch; March 17, nodule felt in epididymis (46 days); March 18, spirochetæ present; March 19, castrated, circumscribed syphiloma size of an olive involving epididymis and testicle. March 29, serum positive, .50 and .15 c.c.; March 30, left testicle slightly enlarged, punctured, spirochetæ not found. April 4, serum negative. 1 c.c.; April 8, left testicle punctured, spirochetæ not found; April 9, serum negative, .1 c.c.; April 11, left testicle punctured, spirochetæ not found; April 15, left testicle: small nodule, spirochetæ not found; serum positive, .1 c.c., 29 days after appearance of lesion in *inoculated* testicle. April 22, left testicle; definite nodule in epididymis, *spirochetæ plentiful.*

The route of infection in this case was probably by the lymphatics, but may have been by the blood, as Truffi (11) has recorded an infection of the anterior chamber of the eye following an inoculation of the scrotum. One of the yaws rabbits has also shown this migration of spirochetæ to the opposite testicle; the lesion developed thirty days after castration of the inoculated testicle.

Treponema pallidum has also been carried through a second and third generation in rabbits.

March 9, grey rabbit, Q, inoculated left testicle, with serum from testicle of Rabbit E (first generation); April 14, nodule in testicle (36 days); April 15, spirochetæ present, April 15, serum positive, .1 c.c.; March 8, white rabbit, L, inoculated with serum from testicle of Rabbit G (first generation) in 2 per cent. citrate solution; April 15, testicle enlarged, nodule, spirochetæ present (31 days); serum negative.

One other rabbit of this generation became infected after forty-three days; three rabbits failed to take the infection.

Three rabbits of the third generation have become infected with an average incubation period of twenty-four days. The fourth generation is under observation. The average incubation period for the first generation was forty-four days, and for the second generation thirty and seven-tenths days.

Treponema pallidum transferred from the Rabbit to the Monkey.—March 9, monkey (*Macacus rhesus* 3): left eyebrow abraded and serum from testicle of Rabbit E rubbed in; spirochetæ present, first generation in rabbit from mucous patch.

April 5, slightly red area on left eyebrow; April 6, lesion very scaly, spirochetæ present (28 days); April 11, flat red area covered with fine scales; April 21, serum negative.

COMPARISON OF THE LESIONS.

The difference in the lesion of syphilis and yaws in the monkey seems to be the surest way to differentiate the organisms. In yaws the incubation period is two to three weeks, the lesion is elevated, slightly scaly, and very edematous; in syphilis the incubation period is about four weeks, the lesion is flat, dry and very scaly. According to Prowazek and Russell, *Treponema pertenui* is slightly thicker than *Treponema pallidum,* less rigid, less regular in its twists. With an abundance of material at hand, comparative observations have been made with the dark field microscope. *Treponema pallidum* seems quite constantly to show more active corkscrew motion; the twists seem more regular and not so sharp and deep; but occasionally an unknown specimen has been classified wrongly on these points and no other differences seem more reliable. The lesion in the rabbit seems to differ only in degree; the nodules in syphilis seem larger and have a necrotic center; histologically, there is evidence of a more active inflammation.

SUMMARY.

1. Rabbits can be infected in the testicle with the spirochetæ of yaws as well as with those of syphilis and the infection can be continued through successive generations in pure culture.

2. The infection shows itself by enlargement of the testicle and the presence of a nodule varying in size from that of a pea to that of an olive. The infection consists in a necrosis of the tubules, an infiltration of round cells and the new formation of an edematous connective tissue.

3. The complement fixation reaction occurs in rabbits infected with the spirochetæ of yaws as well as in those infected with the spirochetæ of syphilis.

4. This lesion makes possible the investigation of the problems of cultivation, of immunity and of treatment.

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EXPLANATION OF PLATES.

PLATE LIII.

FIG. 1.   Colored soldier with yaws; second eruption.

PLATE LIV.

FIG. 2.   Monkey 1, ulcerating yaw on eyebrow and eyelid, 47 days after inoculation from patient.

FIG. 3.   *Treponema pertenui* from Rabbit A, Giemsa's stain, $\times$ 1,000.

FIG. 4.   *Treponema pertenui* in rabbit's testicle, Levaditi stain, $\times$ 1,500.

THE EFFECT OF VAGUS SECTION UPON ANAPHY-
LAXIS IN GUINEA PIGS

BY JOHN AUER

.

THE EFFECT OF VAGUS SECTION UPON ANAPHY-LAXIS IN GUINEA PIGS.[1]

SECOND COMMUNICATION.

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PLATES LV AND LVI.

INTRODUCTION.

In a previous paper[2] dealing with some of the functional disturbances of a type of very acute serum anaphylaxis in the guinea pig, we came to the conclusion that the death of the animal was due to asphyxia caused by a tetanic contraction of the bronchial muscles, the contraction being so pronounced that the lumina of the smaller bronchial tubes were occluded, thus preventing both the entrance and escape of air. This tetanic contraction imprisoned the air in the lung so that this organ could be excised *in toto* with practically no collapse. The evidence for the conclusion stated above was fully considered in this earlier paper[3] and I shall not enter upon this question now; it may, however, be added that this view has since been shared by Anderson and Schultz[4] and by Biedl and Kraus.[5]

On the basis of this assumption, therefore, it became of interest to determine what the effect would be if the bronchial musculature were deprived of its innervation. In this way, perhaps, it could be established whether the toxic injection of serum affects the nerve endings in the bronchial muscles, or the muscle substance directly,

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or both structures. Now it is well-known that the vagus nerve supplies the motor innervation for the bronchial muscles;[6] section of this nerve, therefore, and an adequate lapse of time for degeneration of the peripheral stump would rob the bronchial muscle of its nerve supply, provided that the vagus motor fibers are not preganglionic. This method, therefore, would seem to permit an experimental approach to the problem.

EXPERIMENTS.

The experiments were carried out entirely in guinea pigs, and the members of each series always belonged to the same lot and were approximately of the same age and weight. They were treated exactly alike in the preparation; the same horse serum in the same amount, either one or two cubic centimeters, was injected subcutaneously for sensitization; the vagus nerve was resected in the neck under ether anesthesia, and it was attempted to remove the left or right vagus in an equal number of guinea pigs. After a varying period of time (details will be given later), the toxic dose was always injected into the jugular vein through a canula, the injection being followed by one cubic centimeter of Ringer solution to wash out the canula. For the toxic dose I invariably employed horse serum which had been heated to 55° C. for thirty to thirty-five minutes. The toxic injection was, as a rule, just above the surely fatal dose.

Before entering upon a report of the experimental results, some suppositions, basic for this investigation, must be discussed. From the arrangement of the experiments stated above, it will be seen that two fundamental assumptions are made, which are of vital importance. These assumptions are that the bronchomotor fibers of the vagus do not end in ganglion cell stations, are not preganglionic, in other words; and, secondly, that these motor fibers do not decussate, so that one vagus innervates only the lung on the same side.

For the first assumption, that the bronchomotor fibers of the vagus are not preganglionic, there is sufficient evidence. It is well known that the motor fibers for the bronchial muscles in the neck

[6] For literature see Dixon and Brodie, *Jour. of Physiol.,* 1903, xxix, 115.

vagus remain irritable for a comparatively long time after the death of the animal.[7]   Now, if the motor lung fibers in the vagus were preganglionic, this ought not to be possible, for Langendorff[8] showed that stimulation of *preganglionic* fibers swiftly becomes ineffective after death of the animal by bleeding or by asphyxia, while stimulation of the *postganglionic* fibers will then still yield results.   Langendorff's results have been corroborated and extended by Langley[9] who states that postganglionic fibers give the customary response one quarter to three quarters of an hour after death, while preganglionic fibers soon lose their effectiveness after death.   Langley also states that he has found the same thing true in all sympathetic fibers with which he has experimented.

That the guinea pig forms no exception to the general rule, I have had some occasion to observe accidentally and in experiments made especially for this purpose.   Occasionally, an animal died through some accident or was killed after having been prepared for registration of intrapleural pressure or of lung volume by oncometers.   In these animals stimulation of the vagi still showed distinct and definite changes in the intrathoracic pressure or of lung volumes fifteen and twenty minutes after the heart had ceased to beat (Plate LV, Fig. 2, Plate LVI, Fig. 3)..   From the data submitted, it will, therefore, be seen that there is good evidence for the assumption that there is no cell station between the bronchomotor fibers of the neck vagus and the bronchial muscles themselves.

In this connection it may be observed that no very marked differences between the two vagi, as far as the bronchomotor effect is concerned, were noticed.   This is in agreement with the observation of Einthoven,[10] though other investigators have found that the left vagus usually exerts a greater effect than the right.[11]   Occasionally I have observed animals in which stimulation of one vagus gave only slight results, while the other vagus responded well.   This diminished irritability occurred sometimes in the right, sometimes in the left vagus.

The second assumption was that the bronchomotor fibers of the two vagi do not decussate, that thus each vagus innervates only its

[7] For references see Dixon and Brodie, *loc. cit.,* 115, 152.
[8] Langendorff, *Cent. f. Physiol.,* 1892, v, 130.
[9] Langley, *Jour. of Physiol.,* 1893, xv, 181.
[10] Einthoven, *Pflügers Arch.,* 1892, li, 381.
[11] For literature see Dixon and Brodie, *loc. cit.,* 118–119.

ipselateral lung. Dixon and Brodie experimented on cats, dogs and rabbits and they state explicitly that both the constrictor and dilator fibers in the vagus supply the lung on the same side only.[12] As there were no observations on the guinea pig, I made some experiments to test this question, though it seemed improbable that the guinea pig should form an exception. The results confirm largely the statement of Dixon and Brodie; in only one or two experiments did I find that stimulation of one vagus occasionally exerted a bronchoconstrictor effect upon both lungs (Fig. 2), and in one of these experiments it is noted that the vagus was stimulated low down so that the possibility of current escape to the other vagus must be taken into consideration. Fig. 3 gives a qualitative picture of the usual result obtained. If there is a decussation of vagus bronchomotor fibers, it cannot be an extensive one, and, therefore, need not be taken into serious consideration.

Another assumption made is that all the bronchomotor fibers run in the vagus. I have no experimental data of my own in support of this, but Dixon and Brodie[13] have found that the sympathetic in cats, dogs and rabbits carries no bronchodilator or broncho-constrictor fibers, and it is not likely that the guinea pig forms an exception to this.

The considerations given above show, I think, that the assumptions made for this investigation are reasonably grounded on experimental facts.

RESULTS.

In the *first series* of experiments both vagi were cut in sensitized guinea pigs shortly before the intravenous injection of the toxic dose. The course of the immediate anaphylactic reaction was perfectly typical and ended in death within five minutes. The lung picture was characteristic when the thorax was opened and the organ excised.[14] Section of both vagi exerted no appreciable effect in any way on the onset, course and final outcome of the reaction.

In the *second series* nine guinea pigs were used. They were all

[12] Dixon and Brodie, *loc. cit.,* 172.

[13] Dixon and Brodie, *loc. cit.,* 142, 172.

[14] For a full description of the gross appearance of the acute anaphylactic lung, see Auer and Lewis, *Jour. of Exper. Med.,* 1910, xii, 156.

sensitized on the same day by the subcutaneous injection of one cubic centimeter of horse serum. Thirteen days after sensitization, one to two centimeters of one vagus nerve was resected in the neck; in five animals the left vagus was resected, and in four, the right. The toxic dose was injected thirty to fifty-seven days after vagus section; two animals were utilized after thirty days, two after thirty-one days, three after forty days, and the remaining two fifty-seven days after vagus section. The toxic dose ranged between 0.8 cubic centimeter to 1.5 cubic centimeters of a 10 per cent. solution of heated horse-serum. In six of these animals death occurred under typical symptoms within four minutes; for purposes of convenience, death was considered to occur when the respiration had permanently stopped.

The autopsy picture of the lungs was typical. On opening the thoracic cavity by first cutting away the thoracic attachments of the diaphragm, the lungs were seen to fill the entire cavity and appeared to suffer no appreciable collapse. No difference could be seen between the two sides; both appeared equally large. On total removal of the lungs from the thorax no collapse was seen, and more careful comparison showed that both sides, innervated as well as denervated, possessed about equal volume (Plate LV, Fig. 1). The lungs looked, in short, exactly like those obtained from an ordinary sensitized guinea pig which had succumbed to the toxic injection within five minutes. In two instances the left half of the lungs looked a trifle larger and fuller than the right, this being especially true of the lower lobes. In one of these animals, the left vagus has been resected, and in the other, the right. Therefore, vagus section could not be held accountable for this difference in size. Moreover, I have frequently had occasion since the beginning of this work to see that anaphylactic guinea pigs with intact vagi often show a slight difference in fullness between the two sides of the lungs, and that this fullness occurs more frequently in the left, especially the lower lobe, than in the right side.

In three animals of this series sublethal doses of horse serum were injected and the animals then killed by section of the medulla. The lungs of these animals were full and large, but not to the same degree as in the six other animals. There was again no difference between the two sides of the lungs.

In this series, resection of one vagus exerted no visible effect on the gross appearance of the lungs.

In a *third series,* composed of eight animals, the vagus was resected in four on the right side and in four on the left side fifty-five days after sensitization (one cubic centimeter horse serum subcutaneously). The toxic dose was injected intravenously in three animals six days after vagus section, in two after thirteen days and in the remaining three after fourteen days. In the first three animals the toxic dose employed was 0.6 cubic centimeter heated horse serum; in all the rest 0.3 cubic centimeter of a 10 per cent. solution of heated horse serum was used. All the animals except two died with typical symptoms within five minutes, and these two survivors had received, previous to the toxic dose, a prophylactic injection of atropine.

The lungs on autopsy again showed no difference between the two sides which could be ascribed to the vagus section; they looked like typical anaphylactic lungs, like normal lungs which had been fully inflated and fixed in that position. In two cases the left sides of the lungs looked fuller than the right; in one of them the left vagus had been resected, and in the other the right; all the others (four) showed no appreciable difference in fullness between the right and left halves of the lungs.

In this series, also, vagus resection did not exert any influence upon the production of the typical anaphylactic lung.

In a *fourth series* of animals, the left vagus was resected in four animals and the right in three. All these animals were normal and non-sensitized when the operation took place. Sensitization was performed thirty-three days after vagus section by injecting subcutaneously one cubic centimeter of horse serum. The toxic dose was injected intravenously fourteen days later in five animals and fifteen days later in two. The toxic dose varied for the first five animals between 0.3 and 0.5 cubic centimeter; in the remaining two animals 0.15 cubic centimeter was injected. All except one of the animals responded typically to the second injection of horse serum and died within seven minutes (in one instance only was this time consumed). The exception noted above was due to intravenous injections of atropin. Autopsy demonstrated the typical lung pic-

ture of immediate anaphylaxis[15] in guinea pigs.   In this series
again no characteristic differences between the two sides of the
lung could be seen.   Three showed no differences at all between
the two sides of the lungs (one had the right vagus resected, the
other two, the left vagus); two showed the left lower lobe a trifle
smaller than the right lower lobe (one animal was deprived of the
right vagus, the other, of the left); the remaining animal possessed
lungs in which the left lower lobe was larger than the right lower
lobe (here the left vagus had been resected).   From these data
it is again easily seen that no macroscopic changes in size of the
lungs were observed with any constancy which could be attributed
to interference with the nerve supply.

Atropin.—In a few animals of these series the effect of atropin was studied
for the following reason: according to the prevailing conception of the action
of atropin, this alkaloid in moderate doses acts only on the nerve endings and
not on the muscles. On the assumption (which did not appear very likely to me)
that the sensitization might affect largely the nerve endings, I argued that a
prophylactic injection of atropin[16] might show a striking picture if the animal
were killed, by cutting the cord, for instance, some minutes after the toxic
injection; the innervated lung ought to be collapsed while the denervated lung
side ought to stand up fully distended with air when the thoracic cavity was
opened, if the sensitization affected the nerve endings. The experimental test,
however, did not yield this result. Three out of five animals which received sub-
cutaneously a prophylactic injection of 3 mg. of atropin survived the toxic
injection and were killed 12 to 15 minutes later by cutting the cord; it may be
added that five controls all died within five minutes. Immediate autopsy showed
that the lungs collapsed well as soon as the chest cavity was opened, and that
there was no appreciable difference between the two sides. The atropin had
affected both sides equally, innervated as well as denervated. It may be argued
that 3 mg. of atropin for a guinea pig weighing approximately 300 grm. is not
a small or even moderate dose, for, in proportion, an individual of 60 kg. would
receive more than 500 mg. of this powerful alkaloid. While this method of
obtaining comparable proportionate doses in different species of animals by
utilizing their weight only is not entirely sound, because it ignores too many
other factors which have an important influence in determining the proper dose,
yet it must be concluded that the dose of atropin given to these guinea pigs
was large enough to affect the bronchial muscles directly. It would have been

[15] By "immediate anaphylactic reaction" we designated a type of acute ana-
phylaxis in which the animal usually dies within five minutes after the intra-
venous injection of the toxic dose. See Auer and Lewis, *Jour. of Exper. Med.,*
1910, xii, 153, 172.

[16] Auer and Lewis, *Jour. of the American Med. Assn.,* 1909, liii, 458. This
subject will be more fully considered in the third communication.

useless to test the effect of a smaller dose of atropin, for, as stated above, 3 mg. failed to save two of the guinea pigs from death. This atropin series of experiments, therefore, gave no information about the function of the nerve endings in anaphylaxis.

DISCUSSION.

The results obtained in the various series of animals with one resected vagus all clearly show that an intact vagus innervation of the bronchial muscles is not necessary for the production of the typical lung picture of immediate anaphylaxis. This is especially well shown in the fourth series of experiments where the vagus was resected and allowed to degenerate before the animal was sensitized. In this series sensitization probably occurred after the vagus had completely lost its effect on the bronchial muscles,[17] for the sensitizing dose of serum was injected thirty-three days after the vagus had been resected, and a time period of almost five weeks is, in all likelihood, sufficient for complete functional degeneration of the distal segment of the severed vagus. Moreover, it must be noted that the toxic dose of horse serum was injected after what is practically the minimum length of time necessary for a certain sensitization, that is, after fourteen days. Yet these animals with one vagus resected were as fully sensitized as normal guinea pigs would be after the same sensitizing dose. There was no sign of a slowed or decreased sensitization; on the contrary, it seemed to me as if these animals were perhaps more highly sensitized than normal animals treated with the same sensitizing dose and used after the same lapse of time; these guinea pigs, with one vagus resected, responded promptly and characteristically with as little as 0.3 and 0.15 cubic centimeter of heated horse serum. Be this as it may, there was surely no retardation or reduction in the sensitization caused by robbing the bronchial muscles of their nerve control. It may, therefore, be legitimately concluded that the bronchial muscles themselves, or at least those of the finer tubes, may be sensitized by horse serum after the vagus innervation of these muscles has degenerated.

[17] Dixon and Brodie, *loc. cit.,* p. 141. These authors found that 14 days after section stimulation of the peripheral stump gave out a very weak constrictor effect, while the dilator fibers were still nearly as effective as in normal animals. After 56 days (no tests were apparently made by these authors between 14 and 56 days) no constriction whatsoever could be obtained, nor did pilocarpin give any contraction of the denervated side of the lung.

The same fact is indicated in the second and third series of experiments, but in those experiments the vagus was resected after the animals had been sensitized for thirteen days and fifty-five days respectively.   Now strictly interpreted, these two series show only that the vagus degeneration does not prevent a sensitized guinea pig's lungs from responding properly to the toxic injection.   These two series, however, while not definitely proving the same point which the fourth series established, show other points of some interest.

In the second series of experiments, for example, the vagus was resected thirteen days after sensitization.   The toxic dose was injected thirty to fifty-seven days after vagus section.   As already stated, the normal response of an anaphylactic guinea pig was obtained.   It may, therefore, be concluded that degeneration of the vagus nerve, after sensitization has about reached the lower limit of effectiveness,[18] exerts no marked effect on the response to the toxic injection

In the third series of experiments, one vagus was resected fifty-five days after the sensitizing dose was injected, and the toxic dose was given intravenously six to fourteen days after the vagus section.   The animals behaved normally, that is, they died in the same fashion as normal, sensitized guinea pigs.   But the toxic dose was injected at a time when vagus degeneration was far from complete, and in the first three animals at a time when the bronchodilator fibers were still physiologically active, while the activity of the broncho-constrictor fibers was also surely much impaired.[19]   If the injected toxic dose, therefore, affected the nerve-endings it might be argued that the conditions were favorable for a bronchial dilatation.   This did not occur; the three animals injected after six days of vagus degeneration showed typical anaphylactic lungs after death.   In addition one of these animals had received a prophylactic subcutaneous injection of four milligrams of atropin sulphate, yet this animal showed only some delay in the onset of death, and its lungs

[18] As stated before, 14 days is practically the smallest time interval between sensitizing and toxic doses, the latter intravenous, which must elapse before the typical chain of symptoms ending in death may be obtained with some certainty.

[19] Dixon and Brodie, *loc. cit.*, 141.

revealed no evidence of any collapse. This hypothetical broncho-dilator effect, due to a stimulation of the nerve endings, receives no experimental support from these data.

In all the experiments reported, there are no definite data which point to a sensitization of the vagus motor endings in the lung. This, of course, does not mean that such a sensitization may not occur. The solution of this section of the problem is unfortunately complicated by the fact that muscular sensitization does occur. Now in order to judge of an effect on the nerve endings, we must rely upon the response of the indicator-organ of the nerve endings, the muscle fibers, for it is impossible to observe the nerve endings alone. But if the indicator organ itself responds, independently of the nerve endings, it is readily seen that no answer will be obtained regarding an action on the endings. This is the case with horse serum sensitization in guinea pigs.

SUMMARY.

Depriving the bronchial muscles of one side of the lungs of their motor innervation does not interfere with sensitization nor the production of the typical anaphylactic lung. There is, therefore, direct sensitization of the muscle substance.

Complete degeneration of the vagus nerve, *after* sensitization has occurred, interferes in no appreciable way with the course of immediate anaphylaxis.

Partial degeneration of the vagus nerve, so that the broncho-dilator fibers are still physiologically active, *after* sensitization has taken place, exerts no effect on the symptoms and lung picture of immediate anaphylaxis (very acute anaphylaxis with death after some minutes).

No definite evidence was obtained regarding the function of the motor nerve endings in the bronchial muscles in anaphylaxis.

The two sides of the lungs in immediate anaphylaxis may occasionally be unequal; when this occurs, the left side, especially the lower lobe, is usually fuller than the right side.

EXPLANATION OF PLATES.

PLATE LV.

FIG. I. Typical anaphylactic lungs in a guinea pig. Magnification about 2×. The animal which furnished this lung was sensitized Nov. 17, 1909, by the sub-cutaneous injection of one cubic centimeter of horse serum. The right vagus was resected Jan. 11, 1910. The toxic dose (0.3 cubic centimeter 10 per cent. heated horse serum) was injected into the jugular vein Jan. 24.

It will be noted that the two lung sides appear about equally full. This lung is indistinguishable from normal anaphylactic lungs.

FIG. 2. Both lower lobes of a sensitized pithed guinea pig were placed each in an oncometer, artificial respiration being maintained. The heart stopped beating about five minutes before this tracing was obtained. The expansion and collapse of the lobes were registered by Marey tambours; upstroke = inflation. The upper curve was obtained from the lower right lobe and the lower curve from the lower left lobe of the lungs. Both vagi were cut.

During the time marked by broad, white bands, the peripheral stumps of either the left or right vagus were stimulated. Time = 6 second intervals; l. p. v. = left peripheral vagus; r. p. v. = right peripheral vagus. Petzoldt coil with two Daniell cells.

PLATE LVI.

FIG. 3. Same animal as in Fig. 2, but the animal had been dead about thirty minutes when this tracing was obtained. Interrupted stimulation of the left and right peripheral vagus stumps with the secondary coil pushed home still yielded definite broncho-constrictor effects, localized to that side of the lung on which the vagus was stimulated. Time = 6 second interval. Note long after-effect.

FIG. 1.
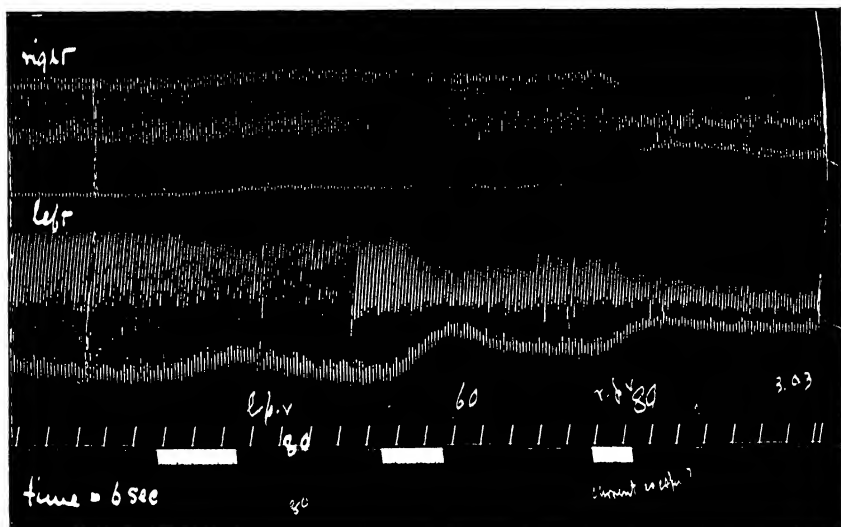
FIG. 2.
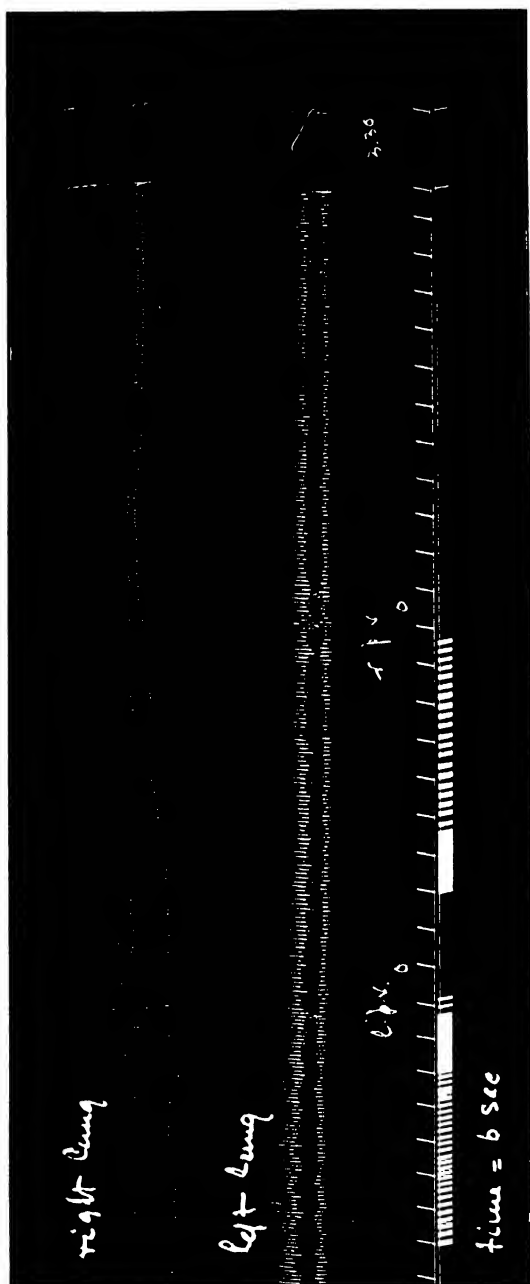
FIG. 3

INTRACELLULAR PROTEOLYTIC ENZYMES OF LIVER

By A. R. DOCHEZ

INTRACELLULAR PROTEOLYTIC ENZYMES OF LIVER.[1]

BY A. R. DOCHEZ.

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In all living tissues, protein constituents are continually being broken down within the body cells and protein decomposition products are excreted. If any or all of the processes of degradation occurring during life are the work of enzymes, we should expect to be able to demonstrate the existence of these enzymes in the tissues after death. The occurrence of such proteolytic enzymes was first established in 1890 when E. Salkowski[2] showed that liver, muscle and suprarenal glands, when minced and kept in chloroform water at 37°C. underwent what he termed "autodigestion." Somewhat similar observations had previously been made by Salomon[3] and by Schmiedeberg.[4] Jacobi[5] later confirmed Salkowski's observations and called the phenomenon "autolysis." These intracellular proteolytic enzymes are not known to be excreted into the body fluids but may be obtained in an active state in the press juice or in the filtrate of water extracts of organs.

Whether the phenomenon of autodigestion of organs removed from the animal body is related in nature to the physiological disintegration of protein within the living cell is a problem that is still unsolved. Parenteral digestion of protein has been observed. The rôle of leucocytes and their enzymes in effecting the removal of inflammatory products has been clearly pointed out by Opie[6] and others. The absorption of areas of necrosis not associated

[1] Presented before the Society for Experimental Biology and Medicine, April 20, 1910. Received for publication July 5, 1910.

[2] Salkowski, *Zeit. f. klin. Med.,* 1890, xvii, Suppl., 77.

[3] Salomon, *Arch. f. Anat. u. Physiol.,* 1881, 361.

[4] Schmiedeberg, *Arch. f. exper. Path. u. Pharm.,* 1881, xiv, 379.

[5] Jacobi, *Zeit. f. physiol. Chem.,* 1900, xxx, 149.

[6] Opie, *Jour. of Exper. Med.,* 1906, viii, 536.

with bacterial infection is probably largely accomplished by enzymes existing in the degenerating cells. Jacobi[7] has been able to demonstrate, in a lobe of liver deprived of its blood supply, the presence of leucin and tyrosin after thirty-six hours. In phosphorus poisoning and in acute yellow atrophy of the liver, protein decomposition products are found in large quantities in the degenerating organ. Such evidence of autodigestion has, however, been observed in pathological conditions in which cell nutrition, oxidation, and other chemical reactions have probably been profoundly disturbed so that an exact analogy to physiological processes cannot be drawn. These facts are nevertheless suggestive, and the possibility is apparent that these extremes of tissue degradation may represent but an intensified picture of normal protein metabolism. In fact, Lane-Claypon and Schryver[8] have been able to show that in hunger, where the animal's energy needs are supplied by his own tissue, autolytic digestion of liver is increased.

The influence of reaction upon autolysis of organs has been exhaustively studied. Investigation of this subject was first carried on by pupils of Salkowski shortly after the discovery of the process of autodigestion. Schwiening[9] has found that sodium carbonate inhibits the autolytic process, and Biondi[10] has demonstrated a favorable influence of hydrochloric acid on autodigestion of liver. Hedin and Rowland,[11] whose investigations were made with tissue plasma obtained by Buchner's method, have established that self-digestion in the majority of organs is facilitated by the presence of 0.25 per cent. acetic acid. It is depressed by the presence of alkalies, of calcium carbonate and of magnesium oxide. Wiener[12] has also demonstrated the sensitiveness of autolytic enzymes to alkali, and has shown that alkalinity corresponding to that of the blood practically checks their power to effect protein decomposition. From this observation he urges that proteolytic endo-enzymes can not be of great physiological importance during

[7] Jacobi, *loc. cit.*
[8] Lane-Claypon and Schryver, *Jour. of Physiol.*, 1904, xxxi, 169.
[9] Schwiening, *Virchows Arch.*, 1894, cxxxvi, 444.
[10] Biondi, *Virchows Arch.*, 1896, cxliv, 373.
[11] Hedin and Rowland, *Zcit. f. physiol. Chem.*, 1901, xxxii, 531.
[12] Wiener, *Cent. f. Physiol.*, 1905, xix, 349.

the normal life of the cell, and autodigestion of tissue in living organisms occurs only in areas in which the blood supply has been reduced.   Such an opinion cannot be regarded as conclusive, since many investigators have observed the power of tissues to digest themselves in alkaline medium.   In addition, proteolytic enzymes acting best in alkaline medium are well known in polymorphonuclear leucocytes, and Hedin[13] has been able to demonstrate the presence of such an enzyme in the cells of the spleen.

Baer and Loeb[14] have tested the effect of varying concentrations of acid and alkali upon autolysis of liver.   Their experiments show the usual favoring influence of acid, and they have obtained a greater relative degree of digestion in alkali than has been observed by other investigators.   They demonstrated an optimum of concentration of alkali in which the degree of autolysis is greater than that found in neutral medium.   Because of this fact, they suggest the possible presence of an alkaline enzyme.   Schryver[15] has found that the favorable influence of acid upon autolysis is dependent upon the absolute amount of acid added and is not specially affected by dilution.   He thinks that acid effects a neutralization of tissue bases which control the activity of the autolytic enzyme.   Hedin[16] has made the interesting observation that after pretreatment of certain tissues, namely, liver, kidney, thymus, spleen, muscle, and testicle, with 0.2 per cent. acetic acid and subsequent neutralization of the acid, autodigestion in 0.25 per cent. sodium carbonate is increased.   Carrying these studies further with the spleen, Hedin has been able to demonstrate an anti-proteolytic action of expressed spleen juice.   This anti-enzymotic action of the juice is exerted against the alkaline enzyme of the spleen, and is destroyed by pretreatment with acid, the increased digestion in alkali after acid treatment being thus explained.

Much interest is attached to the fact that, notwithstanding the presence of destructive agents in all living tissues, organs succeed in guarding their integrity.   In recent years much attention has

[13] Hedin, *Jour. of Physiol.,* 1904, xxx, 155.

[14] Baer and Loeb, *Arch. f. exper. Path. u. Pharm.,* 1905, liii, 1.

[15] Schryver, *Biochem. Jour.,* 1906, i, 123.

[16] Hedin, Festschr. f. Olof Hammarsten, Upsala, 1906, vi, 1.

been centered on the anti-enzymotic properties of blood serum, and of organ extracts. Weinland[17] has shown that the stomach does not digest itself because of the presence of an anti-enzyme in the cells of the gastric mucosa. Intestinal worms resist the action of the digestive juices by means of the anti-enzymes which they contain. Hahn,[18] Landsteiner,[19] Hedin[20] and others have found substances in blood serum which are able to check the proteolytic action of trypsin. Opie[21] has demonstrated that a similar anti-enzymotic power over the alkaline enzyme of the polymorphonuclear leucocytes is possessed by the serum. Many recent studies have shown an increase in the anti-tryptic power of the blood in cachectic conditions, notably in cancer and in chronic pulmonary tuberculosis. The stimulus to the production of anti-tryptic substances has been variously assigned to enzyme freed from disintegrating leucocytes, to pancreatic trypsin and to increased intracellular enzymotic activity, or to combinations of the above factors. Blood serum has, likewise, an inhibiting effect upon the progress of tissue autolysis. The anti-tryptic action of the blood is, however, exerted only against alkaline enzymes such as trypsin and the enzyme of polymorphonuclear leucocytes. Hedin[22] has shown, on the other hand, that blood serum does not effect inhibition when added to that proteolytic enzyme of spleen which acts in acid medium. Since this enzyme is the more conspicuous of the autolytic enzymes the influence of blood serum upon autolysis appears somewhat limited.

Methods.—The tissues used in the following experiments were obtained from healthy normal dogs. Where normal tissue has not been used a special note is made. The animals were anesthetized with ether, and rapidly bled to death from the carotid arteries. The organ used for the study of autolysis was the liver. In certain instances beef serum denaturalized by heat was added to the autolysing mixture as substrate. The autolytic mixtures were in-

[17] Weinland, *Zeit. f. Biol.,* 1903, xliv, 45.

[18] Hahn, *Berliner klin. Woch.,* 1897, xxxiv, 499.

[19] Landsteiner, *Cent. f. Bakt.,* Abt. I, 1900, xxvii, 357.

[20] Hedin, *Jour. of Physiol.,* 1903, xxx, 195.

[21] Opie, *Jour. of Exper. Med.,* 1905, vii, 316.

[22] Hedin, *Jour. of Physiol.,* 1904, xxx, 155.

cubated in the thermostat at 37°C. for five days. Toluol was added in sufficient quantity to prevent bacterial growth. At the conclusion of the period of digestion the coagulable protein was precipitated by boiling with weak acetic acid and twenty per cent. magnesium sulphate. The coagulum was removed by filtering and the incoagulable nitrogen of the filtrate determined by the Kjeldahl method. The amount of digestion is expressed in cubic centimeters of N/10 sulphuric acid. Controls were made in each instance by boiling immediately equal quantities of the materials used and determining the incoagulable nitrogen of the filtrate. The figures given represent the amounts of digestion after the subtraction of controls.

AUTOLYSIS OF NORMAL LIVER IN MEDIA OF VARYING REACTION.

In a number of experiments the autolytic power of liver in acid, neutral and alkaline media was determined. The liver was minced immediately after the death of the animal and the power of self-digestion tested. In some instances denaturalized beef serum was used as substrate. All digestion mixtures were diluted to twenty-five cubic centimeters with 0.85 per cent. salt solution. A few of the experiments performed are cited as examples.

In Experiments I and II the animals were anesthetized with ether and bled to death from the carotid arteries. The liver was aseptically excised without perfusion and rapidly passed through a hashing machine. Beef serum denaturalized by heat was used as substrate. The digestion flasks were incubated for five days at 37°C.

EXPERIMENT I.

Quantity of Liver.	Medium.	Digestion.
2.5 gms.	0.2 per cent. acetic acid	36.6 c.c. N/10 H_2SO_4
2.5 gms.	neutral	6.7 c.c. N/10 H_2SO_4
2.5 gms.	0.2 per cent. sodium carbonate	3.5 c.c. N/10 H_2SO_4

EXPERIMENT II.

Quantity of Liver.	Medium.	Digestion.
2.5 gms.	0.2 per cent. acetic acid	29.4 c.c. N/10 H_2SO_4
2.5 gms.	neutral	7.9 c.c. N/10 H_2SO_4
2.5 gms.	0.2 per cent. sodium carbonate	2.0 c.c. N/10 H_2SO_4

In the above experiments the degree of digestion in 0.2 per cent. sodium carbonate is lower than that usually observed because of the inhibiting influence of the heated beef serum.

In Experiments III to VII no substrate was added.   Otherwise, they resembled Experiments I and II.

EXPERIMENT III.

Quantity of Liver.	Medium.	Digestion.
2.5 gms.	0.2 per cent. acetic acid	20.7 c.c. N/10 H_2SO_4
2.5 gms.	0.2 per cent. sodium carbonate	3.6 c.c. N/10 H_2SO_4

EXPERIMENT IV.

Quantity of Liver.	Medium.	Digestion.
2.5 gms.	0.2 per cent. acetic acid	0.5 c.c. N/10 H_2SO_4
2.5 gms.	neutral	8.7 c.c. N/10 H_2SO_4
2.5 gms.	0.2 per cent. sodium carbonate	4.4 c.c. N/10 H_2SO_4

The total absence of digestion in acid medium in this experiment is difficult to explain, but is obviously referable to a technical error.

EXPERIMENT V.

Quantity of Liver.	Medium.	Digestion.
2.5 gms.	0.4 per cent. acetic acid	35.6 c.c. N/10 H_2SO_4
2.5 gms.	0.2 per cent. acetic acid	34.8 c.c. N/10 H_2SO_4
2.5 gms.	neutral	11.3 c.c. N/10 H_2SO_4
2.5 gms.	0.2 per cent. sodium carbonate	4.8 c.c. N/10 H_2SO_4
2.5 gms.	0.4 per cent. sodium carbonate	0.6 c.c. N/10 H_2SO_4

EXPERIMENT VI.

Quantity of Liver.	Medium.	Digestion.
2.5 gms.	0.2 per cent. acetic acid	23.7 c.c. N/10 H_2SO_4
2.5 gms.	neutral	9.2 c.c. N/10 H_2SO_4
2.5 gms.	0.2 per cent. sodium carbonate	12.2 c.c. N/10 H_2SO_4
2.5 gms.	0.4 per cent. sodium carbonate	10.4 c.c. N/10 H_2SO_4

The liver was carefully perfused with salt solution in the following experiment in order to determine the influence upon autolysis of the small amount of blood left in the vessels after bleeding. The duration of incubation was two days.

EXPERIMENT VII.

Quantity of Liver.	Medium.	Digestion.
5 gms.	0.2 per cent. acetic acid	43.1 c.c. N/10 H_2SO_4
5 gms.	neutral	11.9 c.c. N/10 H_2SO_4
5 gms.	0.2 per cent. sodium carbonate	7.4 c.c. N/10 H_2SO_4

The results obtained in the foregoing experiments are typical of autolysis of normal liver. They confirm the observations of previous investigators for they show the decidedly favoring influence of an acid medium upon the self-digestion of animal tissue. Autolysis progresses much less rapidly in neutral medium, the amount being in most instances less than one-half that observed in acid. As a rule, a still further reduction occurs in alkaline medium, and in some instances the degree of digestion is almost trivial when compared with the corresponding amount in acid. Experiment VII shows that washing out by perfusion the small amount of serum that remains in the vessels of the liver after exsanguination has practically no influence upon the relative amount of autolysis in acid, neutral and alkaline media. Inasmuch as inhibition of autolysis has been observed in expressed tissue juices after thorough perfusion, it is manifestly impossible to free tissue from all inhibiting substances. Experiments V and VI represent two different types of autolytic activity occurring in normal liver. In both instances what is probably the maximal amount of proteolysis is developed in acid medium. In Experiment V the activity of digestion sinks markedly in alkaline medium, and when a concentration of 0.4 per cent. sodium carbonate is reached, no proteolysis occurs. On the other hand, in Experiment VI a second optimum of digestion occurs in alkaline medium and this high degree of autolytic activity is maintained even in a concentration of 0.4 per cent. sodium carbonate. The latter type of activity is rarely observed in autolysis of normal liver. A similar observation has been reported by Baer and Loeb.[23] Inasmuch as it will be shown later that liver contains a masked alkaline enzyme whose activity can be developed by appropriate methods, the result obtained in Experiment VI suggests that the activity of this enzyme is subject to marked variations during life.

An attempt was made to duplicate, in two additional experiments, the second optimum of activity in alkaline media, which I have described above. Normal liver was allowed to autolyze in strengths of acid and alkali varying from one per cent. acetic acid to one per cent. sodium carbonate.

[23] Baer and Loeb, *loc. cit.*

EXPERIMENT VIII.—The liver of a normal dog with no perfusion was excised aseptically and rapidly hashed. Denaturalized beef serum was added as substrate; the duration of digestion was five days at 37°C.

Quantity of Liver.	Medium.	Digestion.
1.25 gms.	0.6 per cent. acetic acid	24.5 c.c. N/10 H₂SO₄
1.25 gms.	0.4 per cent. acetic acid	23.8 c.c. N/10 H₂SO₄
1.25 gms.	0.3 per cent. acetic acid	22.5 c.c. N/10 H₂SO₄
1.25 gms.	0.2 per cent. acetic acid	21.6 c.c. N/10 H₂SO₄
1.25 gms.	0.1 per cent. acetic acid	19.5 c.c. N/10 H₂SO₄
1.25 gms.	neutral	8.2 c.c. N/10 H₂SO₄
1.25 gms.	0.1 per cent. sodium carbonate	8.3 c.c. N/10 H₂SO₄
1.25 gms.	0.2 per cent. sodium carbonate	2.2 c.c. N/10 H₂SO₄
1.25 gms.	0.3 per cent. sodium carbonate	2.3 c.c. N/10 H₂SO₄
1.25 gms.	0.4 per cent. sodium carbonate	2.5 c.c. N/10 H₂SO₄
1.25 gms.	0.6 per cent. sodium carbonate	3.0 c.c. N/10 H₂SO₄

EXPERIMENT IX.—In this experiment no substrate was added to the autolytic mixtures. Otherwise it resembled Experiment VIII.

Quantity of Liver.	Medium.	Digestion.
2.5 gms.	1.0 per cent. acetic acid	35.9 c.c. N/10 H₂SO₄
2.5 gms.	0.6 per cent. acetic acid	34.3 c.c. N/10 H₂SO₄
2.5 gms.	0.4 per cent. acetic acid	35.6 c.c. N/10 H₂SO₄
2.5 gms.	0.2 per cent. acetic acid	34.8 c.c. N/10 H₂SO₄
2.5 gms.	0.1 per cent. acetic acid	31.5 c.c. N/10 H₂SO₄
2.5 gms.	neutral	11.3 c.c. N/10 H₂SO₄
2.5 gms.	0.1 per cent. sodium carbonate	13.0 c.c. N/10 H₂SO₄
2.5 gms.	0.2 per cent. sodium carbonate	4.8 c.c. N/10 H₂SO₄
2.5 gms.	0.4 per cent. sodium carbonate	0.6 c.c. N/10 H₂SO₄
2.5 gms.	0.6 per cent. sodium carbonate	0.5 c.c. N/10 H₂SO₄
2.5 gms.	1.0 per cent. sodium carbonate	0.7 c.c. N/10 H₂SO₄

The results obtained in these two instances are practically identical. A maximum of digestion is obtained in all grades of acid media, but very slight increase occurs in the higher concentrations of acid. An abrupt fall of activity is manifested in neutral medium. In 0.1 per cent. sodium carbonate the amount of autolysis is somewhat higher than that observed in neutral. When a strength of 0.2 per cent. sodium carbonate is reached, another drop in activity takes place and reduces the degree of autolysis to a very small amount. In Experiment VIII no further reduction occurs on increasing the concentration of alkali; in Experiment IX a concentration of 0.4 per cent. sodium carbonate completely checks autolysis. The only evidence in these two experiments of the pres-

ence of an enzyme acting best in alkaline medium is the equal or slightly increased amount of autolysis that is observed in 0.1 per cent. sodium carbonate.

THE EFFECT OF STANDING UPON THE ENZYMOTIC ACTIVITY OF LIVER.

When fresh normal liver is allowed to stand on ice for considerable periods of time, and its power of autolysis determined at varying intervals, a striking increase of autolytic activity in neutral and alkaline media is observed.

EXPERIMENT X.—In this experiment the liver of a dog poisoned by phosphorus was used. The liver was excised aseptically after being thoroughly perfused with salt solution. It was rapidly hashed and diluted with 0.85 per cent. salt solution. Two flasks were prepared; the liver in one was allowed to stand at neutral reaction, and in the other, in a concentration of 0.1 per cent. sodium carbonate. Both flasks were kept on ice. Sufficient quantities of toluol were added to each flask to inhibit the growth of bacteria. Tests of the autolytic activity in acid, neutral and alkaline media were made immediately. Each autolytic mixture contained 0.5 grams of liver; the duration of digestion was five days at 37°C. Beef serum denaturalized by heat was used as substrate. For this experiment I am indebted to Miss B. I. Barker.

The figures represent cubic centimeters of N/10 sulphuric acid.

Liver kept in 0.85 per cent. salt solution at neutral reaction.

Medium.	Duration of period of standing before tests were made.						
	Fresh.	8 days.	20 days.	29 days.	36 days.	44 days.	51 days.
0.2 % acetic acid	11.0 c.c.	13.0 c.c.	10.9 c.c.	11.8 c.c.	10.3 c.c.	10.9 c.c.	9.1 c.c.
neutral	1.9 c.c.	1.4 c.c.	3.5 c.c.	3.2 c.c.	2.3 c.c.	3.4 c.c.	3.6 c.c.
0.2 % sodium carbonate	0.5 c.c.	2.2 c.c.	4.0 c.c.	5.4 c.c.	5.0 c.c.	4.9 c.c.	5.1 c.c.

Liver kept in 0.85 per cent. salt solution in 0.1 per cent. sodium carbonate.

Medium.	Duration of period of standing before tests were made.						
	Fresh.	8 days.	20 days.	29 days.	36 days.	44 days.	51 days.
0.2 % acetic acid	11.0 c.c.	11.0 c.c.	10.3 c.c.	10.9 c.c.	10.5 c.c.		9.1 c.c.
neutral	1.9 c.c.	2.3 c.c.	4.6 c.c.	2.6 c.c.	2.4 c.c.	4.1 c.c.	3.1 c.c.
0.2 % sodium carbonate	0.5 c.c.	1.3 c.c.	1.9 c.c.	2.2 c.c.	2.6 c.c.	3.8 c.c.	4.6 c.c.

EXPERIMENT XI.—Healthy normal dog's liver was used for this experiment. The animal was bled to death from the carotid arteries and the liver was not perfused. Otherwise, the same details were observed as in Experiment X. To one part of the liver, however, sufficient sodium carbonate was added to make the concentration 0.2 per cent. For each test of autolysis 2.5 grams of liver were used. The duration of digestion was five days at 37°C., and denaturalized beef serum was used as substrate.

The figures represent cubic centimeters of N/10 sulphuric acid.

Liver kept in 0.85 per cent. salt solution at neutral reaction.

Medium.	Duration of period of standing before tests were made.					
	Fresh.	10 days.	20 days.	31 days.	45 days.	55 days.
0.2 % acetic acid	29.4 c.c.	20.9 c.c.	23.7 c.c.	20.7 c.c.	20.7 c c.	17.6 c.c.
neutral	7.9 c.c.	16.3 c.c.	9.9 c.c.	22.9 c.c.	17.9 c.c.	21.7 c.c.
0.2 % sodium carbonate	2.0 c.c.	7.6 c.c.	3.1 c.c.	17.4 c.c.	15.0 c.c.	24.2 c.c.

Liver kept in 0.85 per cent. salt solution in 0.2 per cent. sodium carbonate.

Medium.	Duration of period of standing before tests were made.					
	Fresh.	10 days.	20 days.	31 days.	45 days.	55 days.
0.2 % acetic acid	29.4 c.c.	16.9 c.c.	17.5 c.c.	15.6 c.c.	16.4 c.c.	15.1 c.c.
neutral	7.9 c.c.	16.6 c.c.	16.5 c.c.	20.9 c.c.	16.7 c.c.	21.6 c.c.
0.2 % sodium carbonate	2.0 c.c.	4.0 c.c.	1.2 c.c.	11.0 c.c.	7.4 c.c.	13.0 c.c.

EXPERIMENT XII.—The liver used in this experiment was obtained from a normal dog. The animal was bled to death and the liver was not perfused. The method was the same as in Experiment XI, except that in this instance no substrate was added. In each test of autolysis 2.5 grams of liver were used.

Liver kept in 0.85 per cent. salt solution at neutral reaction.

Medium.	Duration of period of standing before tests were made.		
	Fresh.	15 days.	37 days.
0.2 % acetic acid	20.7 c.c. N/10 H_2SO_4	17.7 c.c. N/10 H_2SO_4	14.6 c.c. N/10 H_2SO_4
0.2 % sodium carbonate	3.6 c.c.	11.0 c.c.	15.0 c.c.

Liver kept in 0.85 per cent. salt solution in 0.2 per cent. sodium carbonate.

Medium.	Duration of period of standing before tests were made.		
	Fresh.	15 days.	37 days.
0.2 % acetic acid	20.7 c.c. N/10 H_2SO_4	10.4 c.c. N/10 H_2SO_4	19.8 c.c. N/10 H_2SO_4
0.2 % sodium carbonate	3.6 c.c.	2.3 c.c.	14.6 c.c.

In the above experiments tests of the reaction of the liver solution allowed to stand were made from time to time. The liver

kept in salt solution developed a weak acidity, but in that to which alkali was added immediately, the alkaline reaction was maintained constantly throughout the experiment. At no time was it possible to demonstrate the presence of bacteria; in fact, liver extracts which have been kept on ice for some time have been found to be powerfully bacteriolytic. In all the experiments it is apparent that the addition of acid to the autolytic mixtures of fresh liver develops a maximal activity and the degree of autolysis in acid medium is not increased as the liver stands, but tends rather to diminish slightly. This diminution is more evident in the liver which stands in alkaline solution than in that to which 'no alkali has been added. Immediate autolysis of fresh liver in neutral and. alkaline medium is observed to be relatively low. Upon standing, however, a striking increase in both media occurs until, as is shown in Experiments XI and XII, the degree of autolysis exceeds that observed in acid. The rate of increase of activity is slower in liver kept in alkaline solution than in that kept in neutral medium. The presence of alkali retards but does not prevent the activation of the enzyme acting in alkaline medium. The periods of diminished activity in neutral and alkaline medium observed from time to time are somewhat difficult to explain, but possibly depend upon the development of inhibiting substances during the slow disintegration that proceeds while the liver stands on ice. The increase of autolytic activity in neutral and alkaline medium might be supposed to be due to a slow destruction of the anti-enzyme of the serum, but this destruction is rendered improbable by the observation that the same increase occurs in liver thoroughly perfused, and, moreover, in liver kept continuously in alkaline solution; alkaline reaction not only preserves the serum anti-enzyme, but even increases somewhat its anti-proteolytic power. The increase of proteolytic activity observed in these experiments is analogous to the increase of tryptic activity observed by Vernon[24] in kept pancreatic extracts. This increase Vernon attributes to the slow conversion of trypsinogen into active trypsin. It is probable, therefore, that a like process occurs in kept liver, and normal liver contains two proteolytic enzymes, one acting best in alkaline and the other in acid

[24] Vernon, *Jour. of Physiol.,* 1901, xxvii, 269.

medium. The alkaline enzyme in fresh tissue, however, exists in an inactive state, so that the amount of immediate autolysis in alkali is slight. Upon standing, the inactive enzyme is slowly converted into active enzyme and a corresponding rise in autolytic activity in alkaline medium results. If the acid-acting enzyme exists in the cell also in an inactive form, it is readily activated by the presence of acid and, consequently, even when fresh tissue is used, exhibits its maximal activity in acid medium. It will be shown later in this paper that the enzyme of liver acting in alkaline medium can be activated by treatment with acid. The activating influence of acid upon trypsinogen of fresh pancreas has been known for many years.

THE EFFECT UPON SUBSEQUENT AUTOLYSIS OF PRETREATMENT OF LIVER WITH ACID AND WITH ALKALI.

Hedin [25] has published the interesting observation that treatment of certain animal organs for from sixteen to twenty-four hours with 0.2 per cent. acetic acid increases the degree of subsequent auto-digestion in alkaline medium. This increase he has shown to be due in the spleen to the destruction by acid of an anti-enzyme which holds in check the weak alkali-acting enzyme which is present. In experiments which follow, the methods used by Hedin have been applied with some modifications to autolysis of liver. By these means it has been possible to produce rapidly an increase of the proteolytic activity of the enzyme acting in alkaline medium.

The livers used in the following experiments were obtained from healthy normal dogs. They were excised aseptically and were minced by passing rapidly through a hashing machine. Three flasks of liver were prepared for each experiment and all were diluted with a quantity of physiological salt solution, equal in amount to the number of grams of tissue used. To one flask acetic acid was added until the required concentration was reached, to another sodium hydrate was added to a like concentration, and the third flask was kept at neutral reaction. All the mixtures were allowed to stand for twenty-four hours on ice, and then the acid flask was neutralized with N/10 sodium hydrate and the alkaline

* Hedin, Festschr. f. Olof Hammarsten, Upsala, 1906, vi, 1.

flask with acetic acid. Corresponding amounts of sodium hydrate and acetic acid were not added to the neutral flask because the resulting salt was found to exercise no influence upon autolysis. After neutralization equal amounts of the liver mixtures were withdrawn from each flask and allowed to autolyze in the thermostat in acid, neutral, and alkaline media. In the earlier experiments denaturalized beef serum was added to each flask as substrate, but its use was later discontinued. Toluol was used in all experiments as an antiseptic.

EXPERIMENT XIII.—The liver was obtained from a normal dog bled to death from the carotid arteries. It was excised aseptically without perfusion and passed rapidly through a hashing machine. The liver was prepared for pretreatment as follows: 5 grams of liver + 20 c.c. salt solution; 5 grams of liver + 15 c.c. salt solution + 5 c.c. 1 per cent. acetic acid; 5 grams of liver + 15 c.c. salt solution + 5 c.c. 1 per cent. sodium hydrate.

All the flasks were allowed to stand for twenty-four hours on ice, when the acid and alkali were carefully neutralized, and portions of liver from each flask allowed to autolyze in acid, neutral, and alkaline media for five days at 37°C. Denaturalized beef serum as substrate was used in each mixture. Each flask contained one gram of liver. The figures given represent the amount of digestion after the subtraction of controls.

Medium.	Treated with 0.2% acetic acid.	Untreated.	Treated with 0.2% sodium hydrate.
0.2% acetic acid	14.3 c.c. $N/10$ H_2SO_4	16.6 c.c. $N/10$ H_2SO_4	7.0 c.c. $N/10$ H_2SO_4
Neutral	6.1 c.c. $N/10$ H_2SO_4	4.3 c.c. $N/10$ H_2SO_4	1.4 c.c. $N/10$ H_2SO_4
0.2% sodium carbonate	4.7 c.c. $N/10$ H_2SO_4	3.4 c.c. $N/10$ H_2SO_4	1.0 c.c. $N/10$ H_2SO_4

EXPERIMENT XIV.—The liver of a normal dog was excised aseptically and washed by being thoroughly perfused with salt solution. The liver was prepared for pretreatment as follows: 10 grams of liver + 20 c.c. salt solution; 10 grams of liver + 17.6 c.c. salt solution + 2.4 c.c. 5 per cent. acetic acid; 10 grams of liver + 17.6 c.c. salt solution + 2.4 c.c. 5 per cent. sodium hydrate.

The flasks were allowed to stand for twenty-four hours on ice. The acid and alkali were then carefully neutralized and the liver allowed to autolyze in acid, neutral, and alkaline media with denaturalized beef serum as substrate. The duration of digestion was five days at 37°C. Each flask contained 2.5 grams of liver. The controls have been subtracted from the amounts of digestion noted.

Medium.	Treated with 0.4% acetic acid.	Untreated.	Treated with 0.4% sodium hydrate.
0.4% acetic acid	27.7 c.c. $N/10$ H_2SO_4	37.3 c.c. $N/10$ H_2SO_4	1.3 c.c. $N/10$ H_2SO_4
Neutral	15.6 c.c. $N/10$ H_2SO_4	10.5 c.c. $N/10$ H_2SO_4	10.4 c.c. $N/10$ H_2SO_4
0.2% sodium carbonate	5.6 c.c. $N/10$ H_2SO_4	1.5 c.c. $N/10$ H_2SO_4	9.5 c.c. $N/10$ H_2SO_4

From the liver used in this experiment another series of flasks was prepared in the manner described above. Instead of standing only twenty-four hours at the various reactions, they were allowed to stand for six days before neutralization.

Medium.	Treated with 0.4% acetic acid.	Untreated.	Treated with 0.4% sodium hydrate.
0.4% acetic acid	26.1 c.c. N/10 H_2SO_4	37.1 c.c. N/10 H_2SO_4	0.7 c.c. N/10 H_2SO_4
Neutral	16.3 c.c. N/10 H_2SO_4	15.4 c.c. N/10 H_2SO_4	6.5 c.c. N/10 H_2SO_4
0.2% sodium carbonate	13.5 c.c. N/10 H_2SO_4	3.2 c.c. N/10 H_2SO_4	8.1 c.c. N/10 H_2SO_4

EXPERIMENT XV.—Normal dog's liver was excised aseptically and rapidly hashed. Two series of flasks were prepared. In one series the liver contained what blood remained in the vessels after bleeding, and in the other the cells were carefully washed by centrifugalization with physiological salt solution. Each series consisted of two flasks, the liver in one of which was treated with 0.2 per cent. acetic acid for twenty-four hours on ice, and the other allowed to stand for the same period of time at neutral reaction. The acid flasks were neutralized with N/10 sodium hydrate before the preparation of the autolytic mixtures. Each flask of the latter contained five grams of liver. No substrate was added. The controls have been subtracted. The lower degree of autolysis observed in the washed cells is dependent upon the loss of liver enzyme during the process of washing and centrifugalization.

Unwashed cells.

Medium.	Treated with 0.2% acetic acid.	Untreated.
0.2% acetic acid	45.4 c.c. N/10 H_2SO_4	49.9 c.c. N/10 H_2SO_4
Neutral	31.1 c.c. N/10 H_2SO_4	16.4 c.c. N/10 H_2SO_4
0.2% sodium carbonate	19.7 c.c. N/10 H_2SO_4	17.9 c.c. N/10 H_2SO_4

Washed cells.

Medium.	Treated with 0.2% acetic acid.	Untreated.
0.2% acetic acid	20.2 c.c. N/10 H_2SO_4	26.1 c.c. N/10 H_2SO_4
Neutral	13.3 c.c. N/10 H_2SO_4	12.7 c.c. N/10 H_2SO_4
0.2% sodium carbonate	16.7 c.c. N/10 H_2SO_4	10.2 c.c. N/10 H_2SO_4

EXPERIMENT XVI.—The liver was obtained from a normal dog. It was excised aseptically and hashed without being perfused. Flasks were prepared for pretreatment as follows: 10 grams of liver + 20 c.c. salt solution; 10 grams of liver + 17.6 c.c. salt solution + 2.4 c.c. 5 per cent. acetic acid; 10 grams of liver + 17.6 c.c. salt solution + 2.4 c.c. 5 per cent. sodium hydrate.

All flasks were allowed to stand twenty-four hours on ice, after which the acid and alkali were neutralized. The liver was allowed to autolyze for five days at 37°C. No substrate was added. Each autolytic mixture contained 2.5 grams of liver. The controls have been substracted.

Medium.	Treated with 0.4% acetic acid.	Untreated.	Treated with 0.4% sodium hydrate.
0.2% acetic acid	22.5 c.c. N/10 H_2SO_4	34.8 c.c. N/10 H_2SO_4	3.9 c.c. N/10 H_2SO_4
Neutral	17.2 c.c. N/10 H_2SO_4	12.8 c.c. N/10 H_2SO_4	0.4 c.c. N/10 H_2SO_4
0.4% sodium carbonate	16.8 c.c. N/10 H_2SO_4	7.7 c.c. N/10 H_2SO_4	0.1 c.c. N/10 H_2SO_4

EXPERIMENT XVII.—A normal dog was bled to death from the carotid arteries. The liver was used without being perfused. The flasks were prepared for pretreatment as follows: 10 grams of liver + 20 c.c. salt solution; 10 grams of liver + 17.6 c.c. salt solution + 2.4 c.c. 5 per cent. acetic acid; 10 grams of liver + 17.6 c.c. salt solution + 2.4 c.c. 5 per cent. sodium hydrate.

All flasks stood for twenty-four hours on ice. The acid and alkali were then neutralized and the autolytic mixtures prepared. The duration of digestion was five days at 37°C. No substrate was added. This liver was especially active in alkali when fresh, so that it was impossible to increase to any degree the alkaline activity by acid treatment. Figures representing digestion are given both before and after the subtraction of controls in order to show how the acid-treated liver may apparently exhibit no increase of activity because a high control must be deducted from the observed amounts of digestion.

The figures represent cubic centimeters of N/10 sulphuric acid.

Medium.	Treated with 0.4 per cent. acetic acid.		Untreated.		Treated with 0.4 per cent. sodium hydrate.	
	Digestion observed	Same with control subtracted.	Digestion observed.	Same with control subtracted.	Digestion observed.	Same with control subtracted.
0.2 per cent. acetic acid	25.5 c.c.	14.8 c.c.	28.6 c.c.	23.2 c.c.	6.9 c.c.	0.9 c.c.
Neutral	20.9 c.c.	10.2 c.c.	15.2 c.c.	9.8 c.c.	8.8 c.c.	2.8 c.c.
0.2 per cent. sodium carbonate	24.7 c.c.	14.0 c.c.	18.9 c.c.	13.5 c.c.	9.2 c.c.	3.2 c.c.
0.4 per cent. sodium carbonate	21.4 c.c.	10.7 c.c.	13.6 c.c.	8.2 c.c.	7.4 c.c.	1.4 c.c.

These experiments are in agreement with the experiments of Hedin[26] upon the effect of acid treatment upon autolysis of spleen. In every instance treatment of liver with weak acetic acid has markedly increased the amount of subsequent autolysis in sodium carbonate. The increase of autolysis in alkali is in most instances paralleled by a like increase in neutral medium. The degree of increased activity obtained is dependent upon the initial activity in alkali of the liver when fresh and upon the duration of the time of treatment. Pretreatment of fresh liver with alkali differs from like treatment with acid for it results in the destruction of practically all proteolytic power, whatever the subsequent medium of

[26] Hedin, *loc. cit.*

digestion may be. The increase of activity produced by acid treatment is not entirely due to the destructive action of acid upon the anti-enzymotic power of any serum that may remain in the tissue, for in Experiment XV increase of autolysis in alkaline medium observed in cells thoroughly washed in salt solution is somewhat greater than in those cells which have not been washed. The addition to the autolytic mixtures of denaturalized beef serum causes inhibition of autolysis in alkaline medium. To this thermostabile inhibitory power is attributable the comparatively low degrees of alkaline digestion after acid treatment of liver observed in those experiments in which heated serum has served as substrate. In acid medium, on the other hand, denaturalized serum is actively attacked.

In discussing the increased activity of acid-treated liver in alkaline medium, two other factors must be considered. It has been mentioned that the degree to which the activity of liver in alkali can be increased by treatment with acid is dependent upon the power of the liver to undergo self-digestion in alkali when fresh. Another factor which diminishes the apparent activity produced by acid treatment is the necessity of subtracting a large control referable to autolysis which takes place on ice during pretreatment with acid. Both of these factors are well illustrated in Experiment XVII. The liver in this experiment is more active in alkaline medium than any that has been studied. Consequiently, the increase of activity in alkali produced by acid treatment is comparatively small, and because the difference between the controls of the acid-treated and the untreated liver. is large, entirely disappears when the figures representing controls are subtracted from those representing digestion. Such active livers are, however, rarely observed, and the commonest type of activity is illustrated by Experiment XVI, in which acid-treatment more than doubles the amount of subsequent autolysis in sodium carbonate.

There is some reason to believe that the inactivity following pretreatment of liver with 0.4 per cent. sodium hydrate is not referable to the complete destruction of the proteolytic enzyme, but is due to the fixing of the enzyme in the inactive state in which it

exists within the liver cells.   In Experiment XIV the liver is fairly active in neutral and alkaline media after treatment with alkali, but shows no activity in acid medium after the treatment.   This has been observed in a few other experiments and the possibility that the heated serum added in this instance contained a substance capable of activating the alkaline enzyme of liver was considered, but attempts to confirm this observation were negative.   In fact, all attempts to reactivate the proteolytic enzymes of liver after alkaline treatment have failed.   When fresh pancreas is treated with sodium hydrate there is the same inactivity observed as after like treatment of liver, but this inactive pancreatic extract can readily be activated by the addition of enterokinase.   This fact will be more fully discussed in a future publication.   Furthermore, when liver which has become active in alkaline medium by standing is treated with 0.4 per cent. sodium hydrate, inactivity, which occurs when fresh liver is so treated, is not produced.

From the experiments detailed above, it is obvious that the effects of acid and of alkali treatment on fresh liver are distinctly opposite, the former increasing the proteolytic activity in neutral and alkaline media, whereas the latter diminishes or totally abolishes activity in all media.   The power of alkali to decrease autolysis can scarcely be termed an inhibition of active enzyme but must be looked upon as a preservation of the enzyme which acts in alkaline medium in an inactive condition.   When this enzyme has been activated, for instance, by standing or by acid treatment, weak concentrations of alkali form a favorable medium for its action.

In contrast to the effect of alkaline treatment upon the proteolytic enzymes of liver and pancreas is its effect upon the alkaline enzyme of the polymorphonuclear leucocyte of inflammatory exudates.   The following experiment shows that leucoprotease maintains its activity when kept continuously in alkaline medium and is able to effect proteolysis after treatment with sodium hydrate.

EXPERIMENT XVIII.—Leucocytes were obtained by injecting sterile aleuronat into the pleural cavity of a dog.   The resulting exudate was centrifugalized and the cells carefully washed with salt solution.   One part was treated with 0.2 per cent. acetic acid for twenty-four hours on ice another was allowed to stand in neutral medium, and a third, in 0.2 per cent. sodium hydrate for the same period

of time. The acid and alkali were then carefully neutralized and the digestive power of the cells determined in acid, neutral, and alkaline media. Denaturalized beef serum was used as substrate. The same quantity of cells was employed for each test. The controls have been subtracted.

Medium.	Treated with 0.2% acetic acid.	Untreated.	Treated with 0.2% sodium hydrate.
0.2% acetic acid	10.9 c.c. N/10 H_2SO_4	12.3 c.c. N/10 H_2SO_4	1.2 c.c. N/10 H_2SO_4
Neutral	9.5 c.c. N/10 H_2SO_4	12.8 c.c. N/10 H_2SO_4	8.7 c.c. N/10 H_2SO_4
0.4% sodium carbonate	9.7 c.c. N/10 H_2SO_4	9.7 c.c. N/10 H_2SO_4	5.6 c.c. N/10 H_2SO_4

In this experiment acid treatment has not increased the power of subsequent digestion in neutral medium or in sodium carbonate. Pretreatment with 0.2 per cent. sodium hydrate does not destroy proteolytic activity later tested in neutral and alkaline medium, although the corresponding activity of liver and pancreas so treated is destroyed. The digestion observed in acid medium is referable in part to the activity of the mononuclear cells which Opie[27] has shown contain an enzyme acting in acid medium. Inasmuch as this enzyme is very sensitive to alkali, it is almost completely destroyed by treatment with sodium hydrate.

EXPERIMENT XIX.—The leucocytes obtained from a sterile abscess produced by turpentine were washed as in the previous experiment. One part was allowed to stand in neutral medium and another in 0.4 per cent. sodium hydrate for twenty-four hours on ice. The alkali was then neutralized and the digestive power of the mixtures determined as before. No substrate was added. Each flask contained an equal quantity of cells.

Medium.	Untreated.	Treated with 0.4% sodium hydrate.
0.2% acetic acid	4.7 c.c. N/10 H_2SO_4	1.3 c.c. N/10 H_2SO_4
Neutral	6.2 c.c. N/10 H_2SO_4	7.1 c.c. N/10 H_2SO_4
0.2% sodium carbonate	8.1 c.c. N/10 H_2SO_4	4.9 c.c. N/10 H_2SO_4

In this instance, even though the concentration of alkali was increased to 0.4 per cent., the cells were later able to effect proteolysis in neutral and alkaline media.

From these experiments it appears that leucoprotease is different from the alkaline enzymes of liver and pancreas, for it exists in the cells of inflammatory exudates in an active condition. This

Opie, *Jour. of Exper. Med.*, 1906, viii, 410.

assumption is based upon observations of the effect of alkali upon the proteolytic enzymes of liver and pancreas. It has been demonstrated that alkali tends to preserve the enzyme in the inactive condition, and pretreatment with sodium hydrate renders permanent this inactivity; it·is noteworthy that pancreas can be activated subsequently by a specific substance, enterokinase of succus entericus.

The work described may be summed up in a few general conclusions. Autolysis of fresh normal liver progresses much more favorably in acid than in alkaline medium. When fresh liver is placed on ice in neutral and in alkaline solution, the power of autolysis in neutral and alkaline media, when tested from week to week, increases markedly, whereas autolysis of the same mixtures in acid medium decreases somewhat. The addition of acid to fresh liver develops the maximal autolytic activity in acid medium. Pretreatment of fresh normal liver with weak acetic acid increases the amount of subsequent autolysis in neutral and alkaline media. The enzyme of liver which is capable of proteolysis in alkaline medium probably exists in the cell in an inactive state and requires activation for the development of its maximal activity. If the acid enzyme of liver exists in an inactive condition, it is readily activated by acid which is the best medium for its action. Dog's liver probably contains two proteolytic enzymes, one acting best in acid and the other in alkaline medium. The inhibitory effect of alkali upon liver autolysis is probably referable to the preservation of the inactive state of the alkaline enzyme. The maintenance of the normal equilibrium of the proteolytic enzymes of the liver cell must be intimately dependent upon the preservation of tissue neutrality; inasmuch as we know, on the one hand, that acid reaction means increased activity even if the acidity is subsequently overcome, and, on the other hand, increase of alkaline reaction causes retardation of proteolysis by preventing the activation of the alkaline enzyme. The observation that leucoprotease exhibits proteolytic activity after pretreatment with sodium hydrate suggests that this enzyme exists in an active state in the cells of inflammatory exudates.

I wish to express my thanks to Dr. E. L. Opie for his suggestions and for his interest in the progress of this work.

A TRANSMISSIBLE AVIAN NEOPLASM
(SARCOMA OF THE COMMON FOWL)

By PEYTON ROUS

A TRANSMISSIBLE AVIAN NEOPLASM.[1]
(SARCOMA OF THE COMMON FOWL.)

BY PEYTON ROUS, M.D.

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PLATES LXVI–LXVIII.

Among the many recent observations on transmissible neoplasms are several which may have greatly enlarged our knowledge of tumor behavior and certainly, for the present, have somewhat confused it. The tumors of the lower animals first studied experimentally—those of the rat and mouse—were found to conduct themselves much as do human neoplasms; and results with them rather strengthened than changed our conception of tumor-characters. But there have since been discovered a number of transmissible new growths of unusual behavior, among them a sarcoma of the dog, transmissible at coitus (Sticker, Ewing), an endemic carcinoma of fishes (Plehn, Pick, Gaylord), and a new growth of hares (von Dungern and Coca), transplantable to animals of another species. All of these in their conduct differ more or less markedly from the classical neoplasms, and whether they are to be accepted as genuine tumors is still doubtful. On the other hand, it is possible that our conception of tumor-behavior, based as it is on observations among few species, has been too narrow.

At this time then the discovery and study of transmissible tumors in new species or classes of animals has an exceptional value. And it is for such reason that a sarcoma of the chicken—the first avian tumor which has proved transplantable to other individuals—will here be dealt with in some detail.

New growths are not rare among birds, and those of the common fowl have had attention from several investigators. Fibromas, myomas, lymphomas, carcinomas and sarcomas, some of them with

metastases, have been described. Ehrenreich,[2] and Tyzzer and Ordway[3] have made attempts at transplantation, but without success, if one except an auto-transplantation of a lymphoma accomplished by the latter authors. Ellerman and Bang[4] have shown chicken leukemia to be transmissible, and in some of their animals aleukemic lymphomata resulted from inoculation. But they have also shown, as have Hirschfeld and Jacoby,[5] that the disease is dependent on a filterable virus.

The tumor here reported was found in a barred Plymouth Rock hen of light color and pure blood. It had existed for some two months before the fowl was brought to the laboratory, becoming noticeable when the host was about fifteen months old. The sarcoma described by Ehrenreich and the myxosarcoma of Tyzzer both occurred in adult fowls.

In this hen there was present, projecting sharply from the right breast, a large, irregularly globular mass. It had developed slowly, and without apparent involvement of the health of the host. Operation was done under ether and nearly all of the growth removed. When sliced it was found to have undergone a widespread coagulation-necrosis at the center, but there was a rim of translucent, rather friable, yellowish-pink tissue of glistening, finely striated surface. Macroscopically, the growth suggested a sarcoma. Bits of it were at once inoculated by means of a trocar into the other breast and peritoneal cavity of the host. Like inoculations were also made into two hens from the same setting of eggs. Thirty-five days later the original host was dead of intraperitoneal growths, and in the breast of one of the other fowls, a large nodule had developed. A summary of the protocols follows.

Oct. 1, 1909. *Operation on Original Tumor.*—The fowl bearing the growth is a strong, young hen. The mass is situated on the right breast, in the subcutaneous tissue, and is somewhat movable. It is irregularly spherical in shape, firm, smooth, well-defined, and projects sharply from the breast contour. It

[2] M. Ehrenreich and L. Michaelis, *Zeit. f. Krebsforsch.*, 1906, iv, 586; M. Ehrenreich, *Med. Klin.*, 1907, iii, 614.

[3] E. E. Tyzzer and T. Ordway, *Jour. of Med. Research*, 1909, xxi, 459.

[4] V. Ellerman and O. Bang, *Cent. f. Bakt., Orig.*, 1908, xlvi, 595; *Zeit. f. Hygiene u. Infektionskrank.*, 1909, lxiii, 231.

[5] H. Hirschfeld and M. Jacoby, *Zeit f. klin. Med.*, 1909-10, lxix, 107.

measures 4.8 by 4.1 by 4.1 centimeters. At its outer pole the skin is attached and thinned, with several slight ulcerations over which are dry, dark-red crusts. Elsewhere, the skin is uninvolved, though the subcutaneous fat is thinned.

Under ether most of the mass was to-day excised, a piece 1 by 3 by 0.5 centimeters being left *in situ*. The growth shelled out easily from the surrounding tissue, and was enclosed in a capsule well supplied with blood-vessels.

The part removed is found on section to consist of a rim of translucent, rather friable tissue about a center that has undergone coagulation necrosis and is firm, yellow and opaque. The rim-tissue is glistening, yellowish-pink, finely striated. No debris comes away from it on scraping, and its surface remains smooth. The region of coagulation-necrosis is serpiginous in outline and forms much the larger part of the mass.

Implantation was made by means of a large trocar into the muscles of the left breast of the same fowl and also into the peritoneal cavity. Bits of the tumor-rim approximating 0.03 cubic centimeter were thrust into each site. Similar implantations were carried out on two young hens of the same brood. No cultures were taken at this time.

Nov. 5, 1909. *Autopsy of the Tumor Fowl, Which Died Yesterday.*—The fragment of the original growth is no longer to be found, but in the muscle of the left breast is an ovoid mass 1.5 by 1.5 by 2.2 centimeters, with similar necrotic center and translucent marginal zone. It has no definite capsule and is not sharply separated from the muscle about it. The peritoneal cavity contains about 20 cubic centimeters of a thin, straw-colored fluid. Attached to the lower margin of the liver, to the oblique membrane, and to the parietal peritoneum are many firm, pale yellow, ovoid or globular nodules, the largest about 1 centimeter in diameter. On section these resemble the nodule in the left breast, except that in them the necrosis is irregularly distributed. At the pelvic region, where several of the masses have coalesced, softening and necrosis are extensive. Smears from fragments taken here show no tubercle bacilli; but with aqueous methylene blue a large rod-shaped bacillus, presumably a post-mortem invader, is demonstrable. No growths are present in the other organs.

Nov. 5, 1909. In the muscle of the left breast of one of the young hens inoculated on October 1 is a firm mass measuring 2.5 by 3.5 centimeters. (The remainder of this protocol is given further on.)

Microscopic preparations of the original growth, and of the nodules developing elsewhere in the host on implantation, have shown it to be a spindle-celled sarcoma. The growths from all latter transplantations have yielded similar pictures, so the histology of the tumor will now be taken up.

In a typical section one observes loose bundles of spindle-cells coursing in every direction, and separated from the lesser blood-vessels only by endothelium (Plate LXVII, Figs. 3 and 4). Where such a bundle is cut transversely, the appearance is that of a group of round cells of varying sizes. With Mallory's phosphotungstic

acid stain intercellular fibrils are demonstrable, though they are rare in the more cellular portions of the growth. The spindle cells, while in general large, vary much in size and shape; some are short and plump, some continued into long, slender processes. The nucleus is, as a rule, large and vesicular, with a loose network and several coarse masses of chromatin. Occasionally, it is rod-shaped, and not seldom pyknotic. To the more slender cells, it gives a bulged outline at that region where it is located. Mitosis is fairly frequent and cells with two to five or six nuclei are not rare. These small giant-cells give to the growth here and there a somewhat polymorphous appearance. They are especially to be found where necrosis is beginning. The widespread necrosis seems in general dependent on insufficient vascularization, although hemorrhage from the thin-walled vessels is also occasionally responsible. In some of the later growths a myxomatous degeneration has separated the cells, and in at least one there has followed a liquefaction with formation of a cyst.

The original tumor was better encapsulated than those resulting on transplantation. Indeed, it gave clinically the picture of a benign growth until after its dissemination at operation. The apparent liberation from restraints that took place then is a phenomenon that has been noted by others[6] for the neoplasms of rats and mice. Infiltration has been the rule since. Search shows that it was also present to some extent in the original growth before operation, despite the considerable encapsulation. The tumor cells had pressed through this capsule here and there and invaded the muscle. The muscle fibers in their breaking down furnished on transverse section some remarkable pictures of pseudo-giant-cells (Plate LXVIII, Fig. 5).

Following the growth's successful transplantation, an attempt was made to propagate it further. At this writing, it is in its fourth generation. The results bring out in an interesting way the importance of blood-relationship to the transmission. The tumor has never thrived except in the intimately related fowls of the pure-blood stock in which it was first noticed. The members of this stock were few and their relationship to one another can be stated with considerable accuracy.

[6] L. Loeb, *Jour. of Med. Research,* 1901, vi, 28.

The tumor was found in one of six hens of pure blood from the same setting, and these, with a single cock of pure blood from another source, formed the parent birds of the stock. From them a single generation of chickens had been raised when the tumor was noted. For the first transplantation two of the parent hens (of the same setting as the tumor fowl) were used, but in the later ones the younger generation of chickens was employed. These, which numbered in all only between twelve and fifteen, had at least one parent in common, some of them two, and some may have been the offspring of the tumor hen, or of that in which the neoplasm grew on its first transplantation. It would be interesting to know the exact relationship between these two hens and the young fowls that proved susceptible, but that cannot be ascertained because the eggs were mixed indiscriminately for setting. Yet it is evident enough that the relationship between all of the fowls of the special stock was a very close one.

Out of twelve of this stock to which it has been transplanted, the tumor has grown in three, and they have been the hosts for the tumor generations thus far accomplished.[7] In sixteen market-bought Plymouth Rocks, superficially like the tumor stock but presumably of impure blood, no growth has been obtained; nor has it occurred in five chickens of mixed breed. In two of three market-bought Plymouth Rocks, which were less than three months old, a transient growth, followed by retrogression, was noted (Chart I). In two pigeons and in two guinea pigs the results have been negative.

The transplantation appears to succeed better in young fowls, judging from its partial success in the young, market-bought Plymouth Rocks and its complete failure in adults of the same sort. At present, the tumor, while still growing in hosts of the special stock, is growing slowly; slow growth may be attributable to the fact that no young fowls are available, for all of the chickens of the second generation of this stock are now adult.

Only two fowls thus far have died as a result of the tumor, which attains a large size before the general health is much affected.

[7] Since this was written transplantation to similar fowls of pure blood from another source has proven successful.
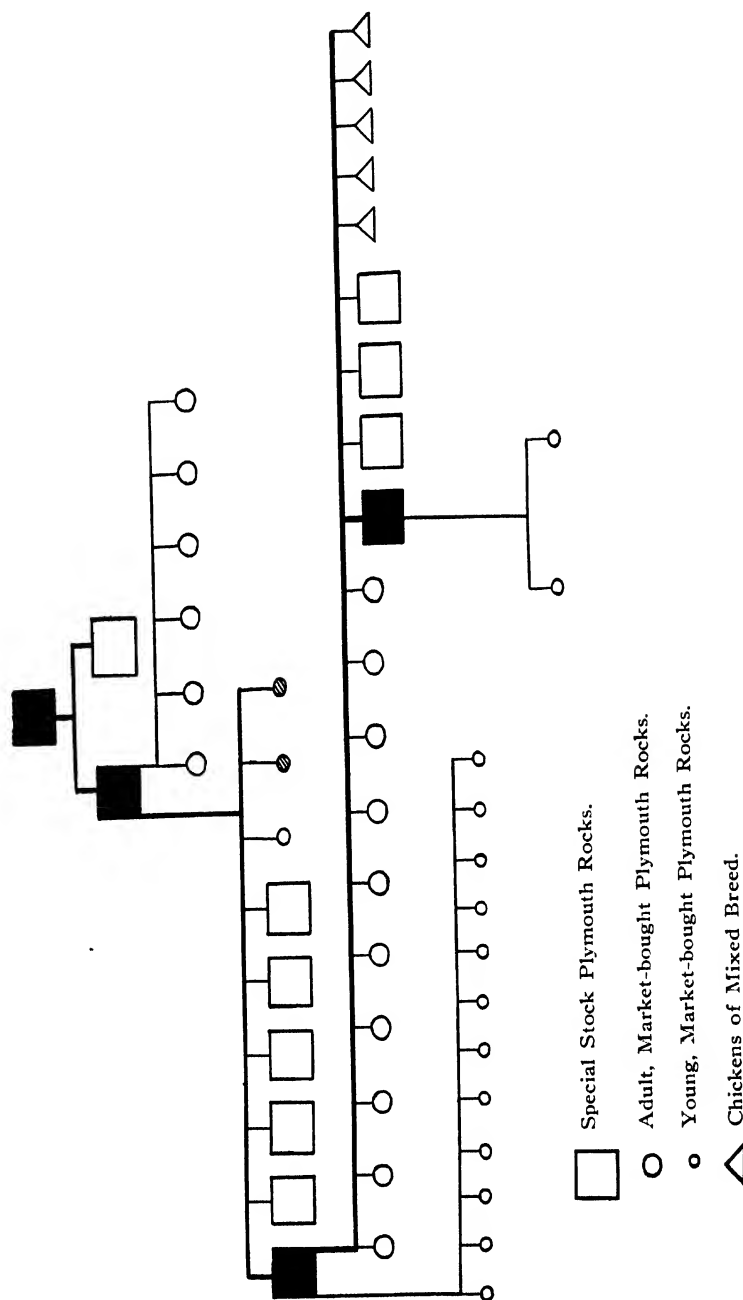
CHART I. Chart showing the course of transmission of the chicken sarcoma. The blackened figures represent individuals in which the tumor grew; the cross-barred ones those in which a nodule appeared but retrogressed.

☐ Special Stock Plymouth Rocks.

○ Adult, Market-bought Plymouth Rocks.

○ Young, Market-bought Plymouth Rocks.

◁ Chickens of Mixed Breed.

(Plate LXVI, Figs. 1 and 2). Thorough bacteriological examinations of the neoplasm have twice been made at operation, but with negative results. Metastasis has occurred once, a tumor nodule being found in the left ventricle of a hen (second generation B) from which the growth had been partially removed some weeks previously (Plate LXVIII, Fig. 6). Whether the operative procedure or a natural dissemination was responsible for this metastasis cannot, of course, be determined. Many intraperitoneal inoculations, and reinoculations of negative individuals have been made, but growth has followed none of these.

A brief summary of the remaining protocols to date will now be given.

First Generation A.—Nov. 8, 1909. One of the 2 young hens inoculated with bits of the original growth was observed 3 days ago to have in the left breast a mass measuring 2.5 by 3.5 centimeters. This was operated upon today and found to be a tumor-nodule. Bits of it were transferred with the trocar to the fowls of *second generation A.*

Dec. 12, 1909. Following operation on the nodule it grew rapidly and to-day measured 5.3 by 4 by 3.5 centimeters, when it was again cut into for the sake of inoculation to *second generation B.* Hemorrhage was so profuse, and the mass was found to extend so deeply into the breast tissue that the fowl was killed. At autopsy, in addition to the mass on the breast, there was present a discrete, firm, translucent, grayish-pink nodule, 0.4 centimeter in diameter, in the substance of the left ventricle. Microscopically, this proved to be a metastasis. The other organs were normal. Fragments of the large growth were placed in bouillon and litmus milk, on sheep-serum agar, plain agar, and Loeffler's blood-serum. Also cultures were taken on gelatine, glucose bouillon, potato, the peptone medium, and glucose agar. These were under observation for several weeks. Bacterial growth occurred in none of them. The Loeffler's blood serum was quite actively digested by the fragment resting on it. Direct smears from the fresh tissue were examined for bacteria, including acid-fast bacilli, but with negative results.

May, 1910. The other animal of *first generation A* has remained free of tumor and healthy to date.

Second Generation A.—Nov. 8, 1909. Six market-bought Plymouth Rock chickens, 2 pigeons and 2 guinea-pigs were inoculated with tumor-bits in the muscle of the right breast and also intraperitoneally. The material was obtained from *first generation A* (q. v.).

Jan. 27, 1910. The chickens were reinoculated, this time into the left breast, with material from *second generation B.*

May, 1910. All are still without sign of tumor.

Second Generation B.—Dec. 10, 1909. Three market-bought Plymouth Rock chickens less than 3 months old, and 6 young fowls, 6 months old, of the tumor

stock, were inoculated in both breasts with bits of the growth from the positive fowl of *first generation A*. The 3 market-bought chickens sickened and died within 12 weeks thereafter. In one was no trace of tumor; in the second was a small nodule (0.6 centimeters in diameter) that had undergone myxomatous degeneration; in the third, a small cyst (1.0 centimeters broad), formed by degeneration and liquefaction of tumor-tissue. Some fragments of this tissue still existed at the well-encapsulated periphery of the cyst.

Jan. 17, 1910. Of the 6 fowls from the tumor stock one had developed at this date a tumor mass 1.3 centimeters in diameter. Jan. 27, 1910. The nodule measured 2.5 centimeters and was operated upon for transfer into *third generation A* (q. v.) and for reinoculation of *second generation A*. At this time many cultures were taken and stains made for bacteria but with negative results. During the next few weeks, the mass decreased in size, and no sign of renewed growth was observed until March 21, 1910. April 7, 1910. Growth has of late been rapid and the mass when today cut into measured 4 by 3 by 2 centimeters. Inoculation was done into *third Generation B*. April 18, 1910, the mass operated upon measured 5 by 3.3 by 2.5 centimeters and was approximately egg-shaped, firm and smooth (Plate LXVI, Fig. 1). The wound had healed perfectly.

In none of the other fowls of this generation was growth obtained.

Third Generation A.—Jan. 27, 1910. Five young fowls of mixed breed, 10 market-bought Plymouth Rocks, and 4 chickens, seven months old, of the tumor-stock were inoculated from *second generation B*.

May 9, 1910. To date none have shown tumors except a cock of the tumor-stock in which was noted March 21 a small lump on the left breast. Operation was performed April 7, and inoculation made into *fourth generation A*.

Third Generation B.—April 7, 1910. Twelve market-bought Plymouth Rocks, under 3 months of age, were inoculated in the muscle of both breasts and in the subcutaneous tissue of the left breast with material from *second generation B*. June 1, 1910. In none of these has growth appeared.

Fourth Generation A.—April 7, 1910. Two of the young fowls inoculated on this date received in the right breast material from *third generation A* instead of from *second generation B*. June 1, 1910. Both of these are negative as regards tumor.

So far as tested this new growth in the chicken has proved itself a neoplasm of classical behavior. The peculiarities which it exhibits are those already made familiar through observations on the tumors of the rat, mouse, dog and man. The tissue specificity which has limited its successful transplantation to fowls of the stock in which the primary growth arose is striking, but not more so than the specificity of certain mouse and rat tumors; and this character may in part explain why previous attempts to transplant neoplasms of the fowl have failed.

The tumor is at best so difficult of propagation that no attempts have been made to determine whether it can be transmitted by cell-

fragments, or by cell-free derivatives. For the same reason, the question whether growth takes place entirely from the introduced cells has not been investigated. But there is no reason to suspect that the neoplasm will differ on these points from the better-known tumors of mammals.

It may not be superfluous to point out that such similarity of behavior as has been thus far observed between this avian tumor and those of mammals was, after all, largely to be expected. A graft of mammalian tumor succeeds better in a blood-related individual, and a young one, not because it is tumor, but because it is tissue. The tissue laws here concerned are probably not very different in birds. Nevertheless, the close correspondence in behavior between this avian tumor and the typical mammalian tumors is certainly of interest. Sticker's lymphosarcoma of the dog, which is transplantable to foxes, von Dungern's tumor of the hare, which will also grow in rabbits, both deviate more from the tumor-type as observed in mammals than does this sarcoma of the fowl. At first sight, indeed, the behavior of these unusual growths seems in absolute violation of the laws governing tissues. Yet this is not necessarily true. For in the hybridization of the horse with the ass, of the dog with the wolf, the elements from different species unite in a much more intimate association than exists between a tumor and its host.

SUMMARY.

In this paper is reported the first avian tumor that has proved transplantable to other individuals. It is a spindle-celled sarcoma of the hen, which thus far has been propagated into its fourth tumor generation. This was accomplished by the use of fowls of pure blood from the small, intimately related stock in which the growth occurred. Market-bought fowls of similar variety have shown themselves insusceptible, as have fowls of mixed breed, pigeons and guinea-pigs. The percentage of successful transplantations has been small, but in the individuals developing a tumor its growth has been fairly rapid. Young chickens are more susceptible than adults. The reinoculation of negative fowls has never resulted in a growth.

Throughout, the sarcoma has remained true to type. It is

infiltrative and destructive. Metastasis has been observed once (to the heart). Experiments to determine whether the growth may be transmitted by cell-fragments have not yet been made. Repeated bacteriological examinations have yielded negative results.

In its general behavior, so far as tested, this avian tumor closely resembles the typical mammalian neoplasms that are transplantable.

EXPLANATION OF PLATES.

Plate LXVI.

Fig. 1. Sarcoma. Second generation B.
Fig. 2. Cross-section of same tumor, somewhat enlarged.

Plate LXVII.

Fig. 3. Sarcoma of the chicken from an intraperitoneal growth.
Fig. 4. Sarcoma of the chicken.

Plate LXVIII.

Fig. 5. Invasion of muscle by the sarcoma.
Fig. 6. A metastasis in the heart wall. The lumen shown at the left hand corner is that of a small vein.

Fig. 1.

Fig. 2.
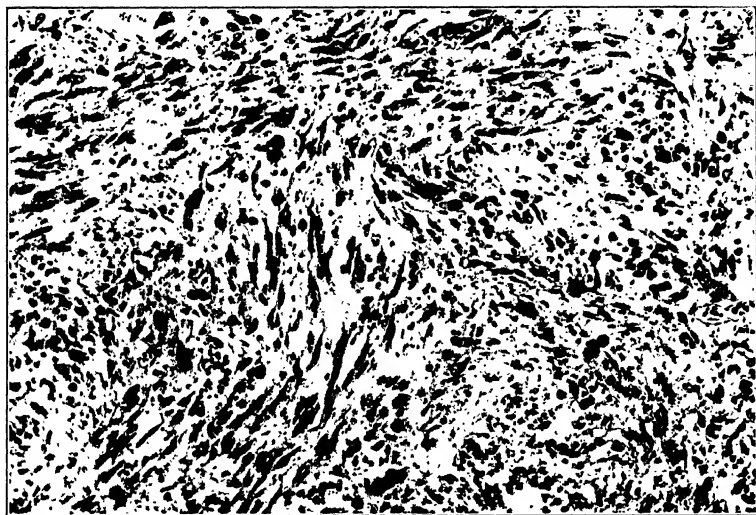
FIG. 3.

FIG. 4.
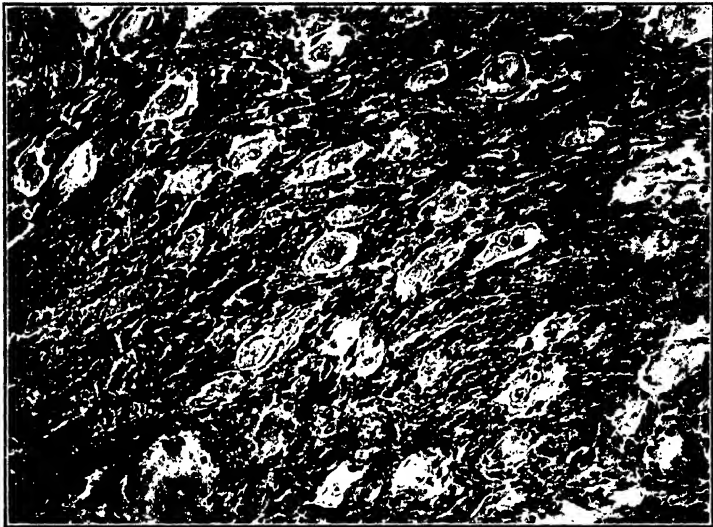
FIG. 5.

FIG. 6.

BRONCHIAL ASTHMA AS A PHENOMENON OF ANAPHYLAXIS

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BRONCHIAL ASTHMA AS A PHENOMENON OF ANAPHYLAXIS

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It is now generally accepted that bronchial or idio-
pathic asthma is due primarily to a stenosis of the
bronchioli. It is not necessary to dwell here on the
particulars of this disease. For the purpose of this
paper, however, the following facts may be mentioned.
Asthmatic attacks occur only in individuals who are
especially subject to it, that is in asthmatics. Further-
more, onset and disappearance of the attacks bear the
character of capriciousness. Heredity, also, seems to be
an influential element in some instances. Finally, there
is practically no pathologic anatomy in this disease.
On the basis of these facts, bronchial asthma is consid-
ered as a functional disease which, according to the pre-
vailing view, means a neurosis; hence also the term
nervous asthma. From the symptomatology we shall
mention here only the following points: By percussion
it can be established that during an attack of asthma the
lungs are overdistended. The auscultation reveals that
there is a considerable decrease of the respiratory ex-
change of air despite the greatly increased efforts of the
respiratory muscles.

There are two theories regarding the nature of the
nervous origin of the bronchostenosis in asthma. One
theory assumes that the stenosis is due to a vasomotor
disturbance, which brings about a swelling of the mucous
membranes or of the submucous tissues of the bronchioli,
causing thereby a narrowing of their lumen. The other
theory assumes that the stenosis is due to a tonic contrac-
tion of the muscle fibers surrounding the finest bronchial
tubes. At present, the latter theory is accepted by most

of the clinical writers, especially since it was established by the careful physiologic investigations of Einthoven[1] and of Brodie and Dixon,[2] that by stimulation of the peripheral end of the pneuomogastric nerves a stenosis of the bronchi takes place which is due exclusively to a contraction of the muscle fibers and not to the production of circulatory changes of any kind. According to this theory an attack is brought on by a stimulation of the broncho-constrictor nerve fibers which run in the pneumogastric nerves. It is assumed that the stimulation takes place, in some obscure way, mostly in the medulla oblongata; in some cases, however, it may be brought about in a reflex way from some peripheral organ. At any rate, asthma, according to this view, is a nervous affection and has its origin in the central nervous system.

It is my intention to attempt to interpret the asthmatic paroxysm as a phenomenon of anaphylaxis, assuming at the same time that the process is a peripheral one. This attempt is based on some very interesting facts recently discovered by Drs. Auer and Lewis[3] in the Department of Physiology and Pharmacology of the Rockefeller Institute. In order to understand clearly the points I wish to bring forward, permit me to make a few elementary remarks on the subject of anaphylaxis or hypersusceptibility.

ANAPHYLAXIS

Anaphylaxis is in an essential point the reverse of immunity. All know what immunity means. Every physician is familiar with the fact that a patient who has recovered from an infectious disease, typhoid, for instance, is less susceptible to a second infection from the same disease. The same applies to the effects of toxins. The experimental work of the last two decades has furnished ample evidence that the injections into an animal of non-fatal doses of toxins, for instance the toxins of diphtheria or tetanus, make this animal more resistant—it immunizes it—against this very toxin. In other words, the passing through a definite infection or intoxication is the means of a decrease of the susceptibility of that animal to the specific infection or intoxication. There were, however, scattered facts which indicated also that

1. Einthoven: Arch. f. d. ges. Physiol. (Pflüger's), 1892, ll, 367.
2. Brodie and Dixon: Jour. Physiol., 1903, xxix, 97.
3. Auer and Lewis: Jour. Exper. Med., 1910, xll, 151. See also THE JOURNAL A. M. A., 1909, lll, 458.

the reverse of immunization may occur; that is, that in-
stead of a decrease of susceptibility, an increase may
occur. For instance, the passing through an attack of
erysipelas seems to prepare the individual for recurrent
attacks of this disease. The same is perhaps also true
of pneumonia. In the extensive work on the immuni-
zation against toxins, several investigators recorded ob-
servations to the effect that an immunization led up to a
hypersusceptibility.[4] However, about five years ago,
striking experiments became known which all at once
raised this issue to a problem of considerable importance.
Our president told us that the present annual meeting
is the twenty-fifth of our Society, and pointed with pride
to the good work which the members have contributed
to medical science. Permit me therefore the historical
remark that the striking and fundamental facts on
which the very interesting chapter of anaphylaxis rests
were first observed and developed by members of our
Association. In the course of his studies of the effects
of diphtheria toxin and antitoxin on guinea pigs, Theo-
bald Smith observed (1903) that by repeated injections
of toxins, these animals instead of becoming immunized
sometimes manifested rather a definite increase of their
susceptibility to the poisonous effects of the diphtheria
toxin. These observations Dr. Smith communicated
orally to Paul Ehrlich during his sojourn in this coun-
try. On his return to his Institute in Germany, Ehrlich
caused one of his pupils, Otto, to investigate systematic-
ally this observation, who, in publishing his results,
designated the fact of the hypersusceptibility as Theobald
Smith's phenomenon. Simultaneously, and independ-
ently of any other observer, the striking phenomenon
was observed and extensively investigated by Dr. Rose-
nau in conjunction with Anderson.[5]

EXPERIMENT

The fundamental experiment which brings out this
phenomenon in a striking manner is as follows:

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4. We may mention here: Behring (1893), Richet (1902), Arthus
(1903), Wolf (Eisner), (1904) v. Pirquet and Schick (1905).

5. The publications of Otto as well as those of Rosenau and
Anderson appeared in 1906, and I am at present unable to state
which one of the publications was ahead of the other. But I can
bear witness to the complete independence of the observations of
Rosenau and Anderson, because in December, 1905, Dr. Rosenau was
kind enough to communicate to me their remarkable experience, and
we discussed the possible meaning of the phenomenon.

A guinea-pig receives a subcutaneous or intraperitoneal injection, let us say, of a small dose of horse serum. The injection produces apparently no effect; the animal remains perfectly normal. When, however, a few weeks later a second injection of horse serum is given to this animal, let us say one c.c. of the serum, given intravenously, the animal dies within a few minutes with striking manifestations. This is an absolutely reliable experiment which never fails. Now you observe that the result as described here was accomplished not with a bacterial secretion, a diphtheria or tetanus toxin, or an endotoxin of any other bacteria, but simply with normal horse serum, which seems to be innocuous in its first injection. In fact, this hypersusceptibility can be produced with such harmless proteins as egg albumin, milk or some vegetable protein, edestin, for instance.[6] A striking feature of this phenomenon is that it is strictly specific; if the first injection was made with horse serum, the phenomenon will occur only when horse serum is used in the second injection, or if edestin was used in the first injection, only a second injection of edestin is capable of producing the fatal phenomenon.

Let me now say a word about the origin of the term anaphylaxis. It was created by Richet some eight years ago. In studying the effects of a poison which he isolated from actinia he distinguished two opposite actions one which produces prophylaxis (immunity) and another which produces anaphylaxis (hypersusceptibility). The term anaphylaxis found general acceptance.

Now let us remember first of all that for the production of anaphylaxis two stages, two separate processes are necessary. The first part consists simply in an injection into the animal body of some foreign proteid. It produces practically no visible effect, but it sensitizes the organism; we call it therefore the sensitizing injection. It need not be an intravenous injection; an incorporation of the proteid in any other manner will have the same effect. The quantity of the proteid is also of no special importance; a very minute dose of the proteid sensitizes an animal. The second part consists, as stated before, in a repeated injection of the same proteid; this is the toxic injection. The effect of this injection is, indeed, most striking, especially when it is given intravenously. The quantity is also of some importance: the larger the dose, the more prompt is the effect. The second injection should not be given too soon after the sensitizing injection; the interval be-

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6. First successfully employed by P. A. Lewis.

tween the two injections should be at least twelve or fourteen days.

The sensitizing effect may be transmitted from the mother to the offspring which, however, may gradually dwindle away. The acquired sensitization leaves a long-lasting impression; even after years a second injection is capable of producing a definite toxic effect. The effect, however, is not always fatal and as striking as described above. When the second dose is not sufficiently large, or when administered in a less effective manner, the guinea-pigs may recover from the "anaphylactic shock" (as the violent attack is often designated), or the attack may set in with less violence.

<div align="center">PHENOMENA OF ANAPHYLACTIC SHOCK</div>

We now come to a discussion of the point which interests us here mostly, namely the character of the attack. From the start, all observers agreed that the death of a guinea-pig in an anaphylactic attack is due to respiratory failure. The attack consists in exaggerated respiratory efforts and manifestations of profound dyspnea. Most of the writers assumed that the cause of this fatal respiratory disorder is located within the respiratory center in the medulla oblongata. Gay and Southard,[7] who have done meritorious work on the problem of anaphylaxis, are emphatic in their claim that the fatal respiratory phenomenon is due to a hyperstimulation of the respiratory center. Although Gay and Southard noticed that the lungs of guinea-pigs dying in anaphylactic shock are "emphysematous" they considered this condition as being only a secondary effect of the violent inspiratory contractions of the diaphragm, the primary cause of the attack being, as stated before, the over-stimulation of the respiratory center.

The recent investigations of Auer and Lewis, however, have in my opinion definitely established the true cause of the anaphylactic shock in guinea-pigs. In the first place, they have established that the lungs, which become greatly distended with the onset of the attack, remain in a distended state even after their complete removal from the thoracic cavity after the death of the animal; in fact, the lungs do not collapse even when cut into pieces. An anatomic study of the lungs demon-

7. Gay and Southard: Jour. Med. Research, 1908, xix, 22.

strated that the distention is due neither to emphysema nor to pulmonary edema. Furthermore, the typical condition of the lungs remained the same even if the second, toxic injection of the serum was administered while the animal was under the influence of curare (and the life kept up by artificial respiration) ; that shows that the distended state of the lungs cannot be simply a secondary effect of the violent contractions of the diaphragm. The real character of the dyspnea was revealed by the fact that during the anaphylactic attack the lungs could not be further inflated by artificial respiration; that is, air could not pass through the bronchi into the alveoli while the respiratory efforts produced at the same time a strong negative pressure in the pleural cavities. 'Finally, Auer and Lewis have established that the anaphylactic attack takes place in animals with a destroyed central nervous system, exactly in the same way as in normal animals, which proves that the process is of peripheral origin. In other words, Auer and Lewis have definitely proved that the cause of the acute anaphylactic death of guinea-pigs is a stenosis of the bronchi due to a peripheral process. This process consists, Auer and Lewis believe, in a constriction of the bronchi due to a tonic contraction of their muscle fibers. It is sure that these contractions are not brought about by central impulses. Whether these contractions are brought about by a stimulation of the muscle fibers themselves or of the nerve endings, this question Auer and Lewis have not attempted to decide for the present. They have discovered the important fact that in many cases the anaphylactic attack can be prevented by a previous injection of atropin. This fact would indicate, according to the accepted view, that the nerve endings are responsible for the attack. On the other hand, Auer[8] recently found that the attack would set in even in animals in which the vagus nerves were cut a long time before the second injection. The nerve-endings therefore must have been degenerated previous to the anaphylactic attack. We may add here that the important results of Auer and Lewis were confirmed by Anderson and Schultz[9] and by Biedl and Kraus.[10]

8. Auer: Proc. Soc. Exper. Biol. and Med., 1910, vii, No. 4.
9. Anderson and Schultz: Proc. Soc. Exper. Biol. and Med., 1910, vii, 32.
10. Biedl and Kraus: Wien. klin. Wchnschr., 1910, xxiii, 385.

The following facts, observed by Auer and Lewis, are of special interest to us here. In an anaphylactic attack the bronchi are constricted; no air can pass into the alveoli, nor can it escape from them; and the lungs are greatly distended and cannot collapse. It was shown that the constriction of the bronchi is of peripheral and not of central origin. Now exactly similar symptoms are met with in so-called nervous asthma. During the attack the bronchi are constricted, air passes through them only with great difficulty. Is asthma a nervous process and of central origin, as is generally assumed, or is it an anaphylactic phenomenon and essentially of peripheral origin? That is, may we not assume that asthmatics are individuals who are sensitized to a definite proteid substance and that the asthmatic attack takes place when the same proteid substance invades the body in the same manner? For one form of asthma this has been established experimentally, and that is for hay fever asthma. As is now well-known, hay-fever is due to the toxalbumins of the pollen of some plants. When such a toxin is injected subcutaneously into a normal individual, it causes no effect whatsoever. If, however, a minute quantity of it is injected into an individual who is subject to hay-fever, in a short time all the symptoms of hay-fever appear, and among them a definite attack of asthma. This can mean only that hay-fever subjects are sensitized to a specific proteid of the pollen of a definite plant, and that whenever the same proteid invades these individuals in some way or other the result is an anaphylactic attack, which, among other phenomena manifests itself in the form of asthma. May we not explain all other forms of asthma in the same manner?

According to this view, to repeat again, an asthmatic is an individual who is sensitized to a definite substance and an asthmatic attack sets in every time this substance manages in some way to enter into the circulation of that individual. We must bear in mind that even in the crude manner of our experiments a very minute quantity of the specific proteid is sufficient for the sensitization as well as for the intoxication. In the actual processes which take place in Nature the effective doses may be infinitely small, and it may well be possible that the minute quantities contained in the emanations from

horses, cats or guinea-pigs are sufficient to act as a toxic
dose and call forth the non-fatal stenosis of the bronchi
evidently present in an asthmatic attack in the human.
Perhaps certain digestion products of the proteins be-
come now and then absorbed into the circulation from
the alimentary canal in some cases in an abnormal stage.

Such an absorption may have little or no influence on
normal individuals, but will call forth an anaphylactic
attack, that is, an attack of asthma, in an individual
sensitized to this protein product. We need not attempt
to work out the details of our theory. Our knowledge
of the anaphylactic process is yet too young and too
scanty for such an undertaking; any application in de-
tail will surely soon have to be rearranged. But with
our theory in mind, a future study of cases of asthma
might bring to light definite causal relations, just as
happened in the studies of hay-fever. For the present,
the following parallel facts may be recalled to mind.
The sensitization to anaphylaxis may be hereditary or
acquired; so is the disposition to asthma either heredi-
tary or acquired. Anaphylaxis is specific; animals sen-
sitized to a definite proteid can be intoxicated only by
that proteid. The same seems to be true also for asthma.
This is certainly true for the hay-fever asthma. Indi-
viduals who suffer from hay-fever in the spring due to
the grass pollen are not subject to the autumnal attacks
which are caused by the ragweed pollen. But the speci-
ficity seems to hold good also for other cases of asthma.
For instance, individuals who get attacks of asthma in
the presence of cats do not get it by the emanations
from horses or from guinea-pigs. A most interesting
and valuable point is the fact that atropin, which re-
lieves asthma, relieves, as it was discovered by Auer and
Lewis, also the anaphylactic attack. On the basis of
the identification of the two phenomena there is an en-
couraging outlook for the therapeutics of anaphylaxis
as well as for asthma. But I shall not indulge in a
further discussion of the details of our subject.

SUMMARY

It is generally agreed that the so-called nervous
asthma is due to a stenosis of the bronchioli. It was
discovered that the so-called anaphylactic shock is due
also to a stenosis of the fine bronchi. The theory is here
offered that asthma is an anaphylactic phenomenon;

that is, that asthmatics are individuals who are "sensitized" to a specific substance and the attack of asthma sets in whenever they are "intoxicated" by that substance.

It has been proved that the anaphylactic attack is of peripheral and not of central origin. It is therefore suggested that the so-called nervous asthma is also due to a peripheral and not a central cause; in other words, "nervous" asthma is not a neurosis.

On account of the capriciousness of the onset and courses of asthmatic attacks, as well as on account of the absence of pathological-anatomical changes in this affection, asthma was considered a functional disease and hence a neurosis. From our point of view asthma is still a functional disease but not a neurosis. We have therefore a significant instance in which a functional disease need not be of nervous origin.

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The Contribution of Experimental
to Human Poliomyelitis

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NEW YORK

THE CONTRIBUTION OF EXPERIMENTAL TO HUMAN POLIOMYELITIS *

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NEW YORK

Epidemic poliomyelitis has within three years become a common and widely distributed disease in the United States. Prior to 1907 the epidemic disease occurred rarely, only, in this country. Since 1907 it has prevailed from the Atlantic to the Pacific Ocean, and probably few states have entirely escaped its ravages. Judging from the fragmentary reports which have come to my attention it would seem as though the southern states had escaped wholly or in large part, but this apparent discrepancy may result from failure either to recognize the nature of the epidemic disease or to report on it, or may be due simply to the circumstance that the fact of its occurrence in the South has not chanced to come to my attention.

It is significant in respect to this point that within the period mentioned epidemic poliomyelitis has prevailed in Cuba. Before 1907 epidemic poliomyelitis had been becoming more frequent in northern Europe, and particularly, so far as reports indicate, in Scandinavia. Beginning in 1907 or thereabout a pandemic of the disease arose. The United States, Austria, Germany and latterly France have certainly participated in the epidemic outbreaks. Whether still other countries have been similarly visited I cannot say, but it is highly probable that they have.

It is a matter of significance that the original foci of the epidemic disease in the United States, occurring in the summer of 1907, were along the Atlantic seaboard,

and the two centers of population most seriously affected were about Greater New York and Boston. The particular point of importance in this regard arises from the fact that those two centers of population receive first and in most concentrated way the immigrant population from northern and eastern Europe. Since, moreover, the best established endemic focus of epidemic poliomyelitis recorded in the last decade or two has been the countries of Scandinavia, the further fact becomes significant that the second large isolated outbreak of the disease in this country occurred in that part of the middle west, namely about Minnesota, which receives a large influx of immigrant population from Norway and Sweden.

CONTAGIOUSNESS

The studies especially devoted to the question of the mode of spread of poliomyelitis, which have been conducted with unusual energy and perspicacity in the last few years, would seem to have rendered contagion highly probable. If the views respecting the manner of the transfer of the contagion put forward with good reason should become established, the explanation of the extension of the epidemic centers of poliomyelitis from northern Europe to America will have become obvious. The data collected in Scandinavia indicate·that the contagion can be carried by intermediate persons from the stricken to the healthy, and from patients not frankly paralyzed, but suffering with slight or so-called abortive attacks of the disease. Moreover, the incubation period of the disease would appear to vary within considerable limits, being sometimes not more than two or three or four days in length and at other times as much as twenty days, the average being eight or ten days, and thus affording opportunity for the transportation across the Atlantic Ocean of the incipiently infected. This particular problem would receive considerable illumination from facts which are ascertainable, such as the number approximately of recently arrived immigrants who developed poliomyelitis in this country since 1907. I am not aware that any effort has been made in the course of the several recent extensive investigations of the epidemiology of the affection to elucidate this important point.

The idea of a contagion in respect to epidemic poliomyelitis is not a new one, but appeared in the literature

more than a quarter of a century ago, and of late has been frequently invoked. The clinical course of the disease indicated an infectious origin, but up to very recent times no convincing knowledge concerning the nature of the agent causing epidemic poliomyelitis existed. Various bacteria, and especially certain cocci, have from time to time been isolated in cultures from fluids obtained by lumbar puncture from patients suffering from the epidemic disease, or from specimens of the central nervous system removed from victims at autopsy. These bacteria did not conform to one species or group of micro-organisms, and did not suffice to set up poliomyelitis in animals. They can be accounted for more satisfactorily as contaminations or secondarily invading bacteria than as the cause of the disease. The epidemic of 1909 in this country, in France and in Germany, led to a renewed study of the nature of the infection, in the course of which the more subtle and recent methods of bacteriology were employed. These methods led to the discovery almost simultaneously in the United States by Dr. Lewis and myself and in France by Landsteiner and Levaditi, that the infectious agent is an extremely minute microorganism that readily passes through the pores of earthenware filters and constitutes, therefore, an example of the so-called filterable viruses, of which at the present time several examples are known to cause infectious diseases in man and the lower animals. The filterable nature of the virus has now been confirmed wherever the subject has been accurately investigated. On acquisition of the fact of the nature of this virus, and of the further fact, on which the discovery of the nature of the virus actually depends, that both the higher and lower monkeys are subject to the experimental disease, rests the recent great advances which have been made in the investigation of epidemic poliomyelitis.

EXPERIMENTS WITH MONKEYS

It was predictable that the effort would be made as soon as circumstances favored to transmit poliomyelitis to monkeys. Since the disease appeared not to be transferable to the more common species of warm-blooded animals, usually available in laboratories, it became imperative to attempt its transfer to other species more nearly related to man. The last few years had indeed

afforded several striking instances in which progress in determining the nature or the conquest of important infectious diseases had resulted from the use of apes and monkeys. Two examples illustrative of this statement are afforded by syphilis and epidemic cerebrospinal meningitis. Hence, in 1907, when the first epidemic appeared in New York and vicinity, we endeavored to transfer poliomyelitis from human beings to monkeys. Unfortunately we were at this time limited merely to fluids obtained by lumbar puncture from cases at different stages of the disease. I say unfortunately for the reason that we had the idea originally of bringing the supposedly infected material directly into relation with the nervous systems of monkeys. This we indeed did with the fluids obtained by lumbar puncture, from which we failed entirely to produce any symptoms that we could discover, including paralysis. During the epidemic of 1907 we did not secure organs from a case of pure infantile paralysis, and we failed, therefore, in our intention to inoculate monkeys from the spinal cord. Had we secured such material the discovery of the nature of epidemic poliomyelitis would, it is fair to assume, have been made two years earlier than it was. It was not until September, 1909, that we·secured the spinal cord from two cases of infantile paralysis in human beings, which specimens were employed for the inoculation of monkeys by direct injection of an emulsion into the brain through a trephine opening. The first inoculations were successful. The animals immediately after their recovery from the ether anesthesia were lively and normal. They remained apparently in perfect health for a number of days, when paralysis set in. The spinal cord derived from these animals was and is still being employed to transmit the infection to still other monkeys.

During the summer of 1909 Landsteiner and Popper published an account of a successful inoculation of two monkeys with the spinal cord derived from a case of infantile paralysis in a child. They employed the peritoneal cavity as the site of injection, .which led to the development of paralysis, but in endeavoring to continue the transfer of the virus by intraperitoneal injection of other monkeys they failed entirely. Strauss and Hun-

toon in this country, who repeated in the late summer
or early autumn of 1909 the experiments of Landsteiner
and Popper, met with the same interruption of their
work. Using the peritoneum as the avenue of entrance
of the virus, they succeeded in producing paralysis in a
monkey inoculated with the human cord, but failed to
produce paralysis when they transferred to other mon-
keys a suspension of the spinal cord of the paralyzed
monkey. In view of these experiments, and of still
other similar ones made by different investigators, it is
probable that the virus contained within the human cord
is on the whole more active when implanted in the mon-
key than is the virus contained within the monkeys'
spinal cords that is derived by proliferation immediately
from the virus of human source. The question for the
moment remains open whether this disparity depends on
the quality of the virus or the number or state of con-
centration of the organisms. Later studies, to which
reference will be made, indicate that the virus of polio-
myelitis is subject to qualitative variations from which
it follows that the sudden change of host might tend to
a reduction in the potency of the virus, the fact of which
reduction is exhibited when the virus is compelled to
traverse a considerable territory and to overcome cer-
tain natural obstacles before it reaches the central
nervous system, on which it became implanted. The
fact of this qualitative change is far less apparent when
the altered virus is brought immediately into relation
with the tissues of the nervous system.

CLINICAL EFFECTS OF INOCULATIONS

In noting the clinical effects of the inoculations we
have paid attention to a number of conditions and signs
that had already been indicated by observations made on
the human affection. We have, therefore, observed an
incubation period represented by the interval elapsing
between the time of inoculation and the appearance of
the first definite paralysis, which incubation period
includes certain manifestations which have been noted
as prodromal symptoms. Thus the shortest period noted
as elapsing between the inoculation and the onset of
paralysis has been three or four days and the longest
period thirty-three days, the average period being eight

or nine days. The prodromal symptoms consist of a state of undue nervousness and excitability on the part of the inoculated monkeys, or inability to fix the gaze, with which is associated a wrinkle and mobile rather than smooth and placid cast of countenance, and an erection of the hairs over the body. These symptoms are most marked for a period of from six to eight hours before the onset of the paralysis. We have not noted any constant elevation of temperature or gastro-intestinal disturbance. The onset of paralysis either when the prodromal symptoms have occurred, or when they have been absent or undetected, tends to be sudden. The paralysis affecting any of the larger groups of voluntary muscles tends to be accompanied with other weak or partially paralyzed groups of muscles. In certain animals the medulla was first affected, and in them death sometimes occurred before the development of actual paralysis. In respect to location, the lower and upper extremities were affected oftener than the muscles of the trunk, and the spinal paralyses were much more frequent than the cerebral. Sensory disturbances occurred, but their investigation was much less satisfactory than the investigation of the motor disturbances. In other words, there is a striking similarity between the frank examples of epidemic poliomyelitis, whether occurring spontaneously in man or produced experimentally in monkeys. A further correspondence exists in this: Slight and evanescent or abortive attacks of the disease have been described in human beings, cases the nature of which would not be suspected were it not for the fact that they occur during the prevalence of epidemics of frank paralysis; and similar abortive or evanescent attacks have been noted among inoculated monkeys, but rarely. There is, however, one important point in which the experimental disease happily differs from the human affection. The mortality in the human affection rarely exceeds 10 per cent. of those frankly attacked, and frequently it is much less than that. The experimental disease, on the other hand, has terminated fatally in about one-half of the first series of animals inoculated, and in a much larger proportion of the later ones. Hence the experimental disease is more highly fatal than the spontaneous disease.

PATHOLOGY

A still further correspondence between the spontaneous and experimental disease is found in respect to the pathologic changes or lesions. The gross lesions visible to the naked eye present in the spinal cord and medulla of monkeys consist of congestion and hemorrhage into the gray matter, chiefly but not exclusively confined to the anterior horns. On the other hand, the general appearance of the spinal cord, medulla and brain are not greatly altered, and the visible effects are no proper measure of the damage inflicted by the virus.

The microscopic lesions are more severe and widespread in the spinal cord than in the brain, and more pronounced in the gray matter and membranes of the cord than in the white matter. No part of the spinal cord, including the medulla, is entirely free from lesions, but the severest lesions tend to occur at levels corresponding to the groups of muscles most severely paralyzed. The meninges show more or less diffuse infiltration with round cells, the greatest accumulations of which are about the blood-vessels, where thick collars of cells often exist. The infiltration is within the adventitial coat, while the muscular coat and the intima remain intact, although the lumina of the vessels are often encroached on through compression. When the vessels are small the effect on the lumina, and hence on the permeability, are considerable. Meningeal cellular invasion is always interstitial and does not give rise to exudate on the surface of the cord or brain, and it is, moreover, made up almost exclusively of mononuclear cells.

The gray matter of the cord shows lesions of the anterior and posterior horns and the commissure, but the anterior horns are as a rule more severely and widely injured than the posterior horns. The chief lesions surround the vessels and consist of a cellular infiltration and edema of the perivascular spaces, and sometimes of hemorrhages as well. When the nerve cells and ground substance are injured, as is frequently the case, foci of similar cells occur there, and the nerve cells show degeneration and necrosis. The extent of the lesions in the gray matter varies greatly. Sometimes minute foci of injury and sometimes complete degeneration of the anterior

horns occur. The infiltration of the perivascular sheaths
of the vessels is continuous with that of the pia-arachnoid.
The white matter of the cord holds in respect to the
frequency and severity of the affection an inferior
position, and the lesions when present there consist of
edema, perivascular cellular infiltration, hemorrhage and
necrosis of tissue. The brain shows lesions that are,
however, more sparse than in the spinal cord. They cor-
respond with cellular infiltrations of the meninges sim-
ilar to but less in amount than in the cord. The inter-
vertebral ganglia regularly are the seat of a diffuse and
nodular infiltration with lymphocytic cells, which collect
between the nerve cells and about the nerve fibers, both
of which may be the seat of degeneration or of necrosis.

The pathogenesis of the affection is explained by the
nature and distribution of the lesions. It would appear
that the virus becomes implanted on the leptomeninges,
especially in the region of the spinal cord and medulla,
where it sets up cellular infiltrative changes that are
most marked in the perivascular lymph spaces of the
arteries entering the nervous tissues. The vascular
lesions constitute the primary causes of the lesions of
the nervous tissue, the severity of which is determined
by the particular vessels affected and the intensity of the
involvement. The infiltrative lesions are confined to
the perivascular lymph sheath and adventitia, but still
other lesions must occur in the intima of the vessels
from which the edema and hemorrhage arise. The cen-
tral arteries entering the anterior median fissure and
supplying the anterior gray matter of the cord invariably
become affected, through which the preponderance of
lesions of the anterior horns is accounted for. Since the
arteries supplying the posterior gray matter are less
important, the lesions in the posterior cornua are
slighter. The degree, therefore, of affection is deter-
mined by the richness of the arterial blood supply,
whence is explained the liability of the lumbar and
cervical enlargements to severe lesions. Irregularity in
the branching of the central artery probably explains the
common variations observed in the involvement of the two
lateral halves of the body. The brain is far less com-
monly the seat of lesions, but it is not spared. Paralysis
of the cranial nerves, and especially of the facial nerve,
follows on them, but lesions also occur in parts of the

brain which do not respond by paralysis. The brain injuries, like those of the cord, depend on vascular lesions.

Hence it would appear that there are good grounds for believing that a considerable part of the paralyses, especially those that are not permanent, are the effects of temporary vascular impediments. The impediments are all outside the lumina of the vessels, which are merely reduced in caliber through pressure. Thrombi do not occur. Some of the functional disturbances are possibly thus anemic in origin; others are probably caused by slight degenerations, and still others are undoubtedly caused by focal hemorrhages and edema. All these effects may possibly be recovered from: part by resolution of the cellular vascular infiltrate and reestablishment of the lumen; part by absorption of edema and hemorrhage, and part by restoration of the mildly degenerated nerve cells. The severer degenerative and other lesions, through which actual necrosis is produced, do not become restored. On them depend the permanent paralyses and deformity.

THE VIRUS

The virus causing epidemic poliomyelitis has been stated to be of very minute size. It is, so far as we can now judge, one of the most minute organisms known to cause disease. This conclusion follows from the fact that in aqueous suspension, such as is secured through preparing an emulsion of the spinal cord in distilled water, it passes with great readiness and little or no loss of potency through the pores of the densest and finest porcelain filters, namely, the so-called Chamberland filter. It passes with even greater ease through the somewhat less dense Berkefeld filter. It is extremely doubtful whether the virus has actually been seen. On staining film preparations of the filtrate with mordanting dyes, preparations are secured which under the highest powers of the microscope exhibit minute points, circular or slightly oval in form, which possibly, although not certainly, represent the stained parasite. When the filtrates are examined under the dark-field microscope, innumerable bright dancing points, devoid of definite size and form, and not truly motile, can be discerned. That

these particles represent the micro-organism of poliomyelitis cannot be affirmed, since similar particles are present in filtrates obtained from nervous and other tissues which can be viewed also as consisting of simple protein matter.

The filtrates are highly potent. Quantities as small as one one-thousandth to one one-hundredth of a cubic centimeter suffice to cause paralysis in monkeys after the usual incubation period, when injected into the brain. The virus is highly resistant to external agencies and conditions. It withstands glycerination for weeks or months very much as the virus of vaccinia or rabies does. It withstands drying over caustic potash for weeks without any or marked reduction in potency, showing·a greater degree of resistance than the virus of rabies. It retains its virulence apparently unimpaired for weeks, on being kept constantly frozen at minus 2 to 4 C. It also withstands for a long time temperatures slightly above the freezing point of water, in the course of which the nervous tissue containing the virus undergoes autolysis, and it has been shown to survive the growth of ordinary mould. On the other hand, it is readily injured by heating, since temperatures of 45 to 50 C. maintained for half an hour suffices to render the filtrate incapable of causing paralysis. It is also readily destroyed by 1 per cent. solution of hydrogen peroxid, and by such simple disinfectants as menthol.

That the virus is a living organism must be concluded from the fact that such minute quantities of it suffice to carry infection through an indefinite series of animals. We have propagated the virus now through twenty-five generations, representing twenty-five separate series of monkeys, and as many removes from the original human material supplying it, and the activity of the virus for the monkeys has increased rather than diminished in the course, and as the result of the successive transplantations. Whether the virus has been or is to be cultivated outside of the body is still an undecided question. We early secured certain indications which led us to hope that the virus multiplied in a medium of bouillon mixed with human serum. We have, however, not succeeded in producing paralysis by the inoculation of one of these possible cultivations. On the other hand, original virus has been observed to retain its virulence for several weeks when kept in a similar bouillon at the temperature of the thermostat.

PROGNOSIS

At the outset of the experiments we estimated that less than one-half of the monkeys that became paralyzed would, if permitted, recover more or less completely from the paralysis. Since, however, our purpose was best served at the time by sacrificing the paralyzed animals immediately on the appearance of the paralysis, they were as a rule etherized. We now believe that the deduction was erroneous and that the fatalities would have been greater than we supposed, and probably as many as three-fourths of the paralyzed animals would have succumbed to the disease. At this early period, however, a number of paralyzed animals recovered, usually, however, incompletely, retaining residues of the paralysis similar to what is observed in the spontaneous human affection. The indications now are that the virus has altered qualitatively and so increased in potency in the later generations that recoveries are hardly to be looked for. The mortality at present approaches 100 per cent., with which figure there is happily nothing in the pathology of the human affection that is comparable. In view of certain experiments of a therapeutic character which are to be mentioned, the fact of the intense activity of the virus should be borne in mind. Undoubtedly examples of the virus will be discovered which will become modified in the reverse direction and lose rather than gain in activity. At one period, indeed, in the course of propagation of each of the two viruses which we originally secured there occurred what appeared to be a sharp decrease of virulence, a change indicated by feeble effects during several passages and the final loss of power of certain strains to transmit the infection. To the circumstance that we carried the virus forward in parallel series of inoculations is to be attributed the survival and increasing activity of the strains at present in use.

INOCULATION OF OTHER ANIMALS

Repeated attempts have been made to implant the virus on other animals, but without success.[1] The animals thus employed consisted of the available warm-

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1. Krause and Meinicke, in Germany, of all those engaged in the recent investigations, alone claim to have transferred the disease to rabbits.

blooded domestic animals of this country, and included guinea-pigs, rabbits, rats, mice, dogs, cats, sheep, cows; goats, pigs, chickens, pigeons and horse. The rabbits and guinea-pigs were inoculated directly with each of the two specimens of human virus, and additional rabbits and guinea-pigs and other animals with virus derived from monkeys. In contrast with these failures is the successful employment of several species of monkeys. The greater number employed were of the species *Macacus rhesus,* but all other species of old world monkeys seem equally susceptible. These included, beside *M. rhesus, M. Cyonomolgus* and *nemestrinus, Cercocebus fulignosus, Cercopithecus callitrichus,* and *Papio babuin.* Of the new world monkeys we employed two species, one belonging to the genus *Cebus* and the other including *Capuchinus.* It chanced that the larger ring-tail proved susceptible and the smaller did not, so that a question arises whether the catarrhine are not more uniformly susceptible than the platarrhine species. No instance of the spontaneous transfer of the virus from a paralyzed to a normal monkey arose, although many opportunities for contagion occurred in the course of our many experiments. This fact does not militate against the notion of contagion in respect to the spontaneous disease in man, for the reason that the monkey is obviously under ordinary conditions and because of the possession of adequate external means of defence an insusceptible species, although once these defences are surmounted it proves less able to resist the injurious effects of the virus than human beings.

MODES OF INFECTION

We have seen that the intracerebral mode of infection is not the only successful one, and that the virus may be introduced into the body by way of the peritoneal cavity, under circumstances leading to paralysis. It has been proved that the virus, when derived from an infected monkey, may also be introduced successfully by way, not only of the peritoneum, but also by means of the general blood, the subcutis, spinal canal, large nerves and certain mucous surfaces. Thus the introduction of large quantities of virus into the stomach or duodenum does not lead to paralysis unless the motions of these organs are for a time arrested by means of opium. When peristalsis has thus been prevented infection and paralysis

result in some instances after this mode of introduction of the virus. However, it would appear that none of the avenues mentioned lead so uniformly to paralysis as does the direct or intracerebral mode of inoculation into the nervous tissues. There exists, however, one mucous surface that is more readily traversed by the virus than the other avenues, excepting the brain, and that is the mucosa of the nasopharynx. If this mucous membrane is lightly scarified in an etherized animal, and the virus rubbed into the scarifications by means of a swab, infection and paralysis usually, and with few exceptions, results with promptitude. Our experience is to the effect that the tracheal mucosa and the lungs do not afford an easy point of entrance of the virus into the body, at least under conditions in which gross lesions of the superficial tissues have not been previously produced.

The facts just given concerning the several possible portals of entry of the virus of poliomyelitis into the body under conditions leading to paralysis have significance with respect to the usual portal of entry of the poison in the spontaneous infection in man. We have been led by certain theoretical considerations to view the nasopharynx as the location in the body to be regarded with special suspicion as being the portal of entry of the virus. Some of these theoretical considerations may be mentioned. Our attention was arrested by the frequently observed fact that in point of distribution epidemic poliomyelitis resembles epidemic cerebrospinal meningitis, and that the two diseases, indeed, so often presented such close similarities in this respect that they had often been confounded with each other. The chief and striking difference between them related to the seasonal prevalence, which for epidemic poliomyelitis is midsummer and for epidemic cerebrospinal meningitis late winter or early spring. The two diseases, moreover, attack by preference infants and young children, although not sparing older children and adults, and in about the same ratio. In the majority of instances a single case appears in a family or home, but often two cases, and less often three or more cases appear. The relation between the group cases in a house or locality has in respect to both diseases been made out only recently, through the discovery of the rôle of the intermediate carrier of the infection.

Now it is held that the *Diplococcus intracellularis* passes into the cerebrospinal membranes by way of the lymphatic connection existing between them and the nasopharyngeal mucous membrane. None of the lower animals, not even monkeys, are so susceptible to the pathogenic effects of the *Diplococcus intracellularis* that its inoculation into the nasal mucosa suffices to set up acute meningitis. We have already seen that the virus of poliomyelitis, which is so much more active, can be introduced successfully by direct inoculation into this membrane.

The considerations thus far given show the close agreement existing between the two diseases, epidemic meningitis and epidemic poliomyelitis, in respect to the part played by the nasopharyngeal mucous membrane. Still other considerations are these: It is difficult, if not impossible, to establish in human beings the fact that the diplococcus passes from the meninges by a reverse lymph current into the nasopharynx, and yet such a migration is not only highly probable, but would most readily and satisfactorily explain the persistent intracellularis infection of these mucous membranes, which is regularly present in epidemic cerebrospinal meningitis. The case is quite different in monkeys infected with *Diplococcus intracellularis* by injection of cultures into the lumbar spinal canal, in which the migration into the nasopharynx of the diplococcus contained in leukocytes, and free also, has been followed with the microscope. It may therefore be regarded as established that this mucous membrane serves both as the site of escape from and of entrance into the meninges of the *Diplococcus intracellularis* in man. The question arises: Does this membrane serve a similar double function in respect to the virus of poliomyelitis? That it may serve for its entrance into the body we have already seen, and the experiment is readily made to determine whether or not the virus is also excreted there. The excised mucosa of monkeys recently paralyzed and killed has only to be rubbed up with quartz sand, suspended in distilled water and pressed through a porcelain filter in order to secure a fluid free from bacteria and suitable for inoculation into the brain of healthy monkeys. By employing this method we have been able to produce paralysis, and thus to prove that the mucous membrane contains the virus. The virus in the mucous membrane is not derived from

the blood contained within it. It is true that the blood
does contain the virus, but in such minimal quantities
that an amount of two cubic centimeters may fail to
cause the infection, while as much as 20 has caused
typical paralysis. Moreover, we have ascertained that
the organs generally do not contain the virus in such
amounts as readily to convey the infection. We have
failed repeatedly to secure infection from the spleen,
bone marrow, liver and different groups of lymphatic
glands, and in several experiments failed to develop
paralysis after injection of the salivary glands, although
Levaditi has reported one such successful experiment
among several failures. We have failed to produce
paralysis by the injection of a suspension of the mesen-
teric lymph nodes taken from an animal that had become
paralyzed as a result of an intrastomachic injection of
the virus, while we have been successful in producing
paralysis with the mesenteric lymph nodes secured from
a human case of poliomyelitis. That the lymphatic
glands in the monkey are capable of retaining the virus
in an active state is proved by the fact that after sub-
cutaneous inoculation of an emulsion of the spinal cord
derived from a paralyzed animal, the regional lymphatic
glands (axillary and inguinal) proved infectious.
Again, Römer and Leiner and Wiesner have in certain
instances caused paralysis by injecting into the brain
emulsions of the mesenteric and of the cervical lymphatic
glands taken from monkeys which became paralyzed fol-
lowing an intra-cerebral inoculation of the virus. It
appears, therefore, that the virus may be retained for
a time by certain distant organs, and especially by the
lymphatic glands, which it reaches through the general
circulation; but these localizations give no indication of
the point of entrance into the body of the virus. The
virus has thus far not been found in the excreta, that is,
in the feces and urine, nor in the intestinal mucosa or
bile. Since the virus is filterable it is possible to demon-
strate its presence in material highly contaminated with
bacteria.

We are disposed to the view that the nasal mucosa
serves not only as the portal of infection but also as the
path of elimination of the virus into external nature,
since such elimination must occur in order that the virus

be maintained alive and transmissible. It is well known
that direct connections exist between the meninges and
the nasal mucosa by way of the lymphatics, which pass
with the filaments of the olfactory nerve through the
cribriform plate. We have already alluded to the fact
that the virus first becomes implanted on the leptomen-
inges, and this primary location of the virus is what
would be especially favored by the mode of entrance of
the virus just mentioned. That the early participation
of the leptomeninges does not occur without certain
definite corresponding alterations taking place in the
cerebrospinal fluid we have been able to demonstrate.
It will be recalled that the cerebrospinal fluid obtained
by lumbar puncture from cases of the human spon-
taneous disease at different periods after the onset of
paralysis, have shown only slight qualitative abnormali-
ties. These fluids, which are clear, but may be in excess,
show merely a moderate increase of the lymphocytes
normally present, and no other qualitative changes.
Moreover, this fluid has been shown by repeated tests to
be non-infectious, or at least not capable of setting up
paralysis in monkeys, even when injected into the brain.
In monkeys on the other hand, in which the spinal fluid
can be investigated during the incubation period of the
disease, as well as after the development of paralysis, it
has been shown that the reaction of the meninges to the
injection of the virus occurs quickly and that at the
expiration of periods of twenty-four, forty-eight or
seventy-two hours, the number of cells within the fluid
has progressively increased, so as to give rise to a slight
opalescence of the fluid which becomes subject also to
spontaneous coagulation. The fluids contain an excess
of protein, as may be shown by means of Noguchi's
butyric acid test. This condition of increased cells and
protein is of brief duration and is succeeded by one in
which the fluid is clear and non-coagulable and the pro-
tein not increased, but only the lymphocytes are more
numerous than normally. This is the state of the fluid
in monkeys at a period concurring with the onset of
paralysis, or appearing soon after, so that there is essen-
tial agreement between the two conditions. The spinal
fluid taken from monkeys when clear and limpid, does
not convey the infection and produce paralysis, but when
the fluid is taken at an earlier period, at the height of

the opalescence, it is capable of transmitting the disease.

Finally, the question arises whether the virus of poliomyelitis can be directly implanted on the leptomeninges, which question can be answered in the affirmative. If an active virus is introduced by lumbar puncture into the meninges, infection and paralysis may be produced. Hence it would appear as though all the theoretical conditions required to establish the nasal and meningeal route as a direct one for infection in poliomyelitis had been supplied by experiment.

It remains to add that in course of the demonstration given by these experiments, two important subsidiary problems have been elucidated: First the pathogenesis of the affection has been rendered clear and comprehensible, and next the changes in the spinal fluid induced by the infection have been brought clearly into view as constituting criteria on which, even in human cases, an early diagnosis of the disease, preceding the onset of any meningeal or paralytic symptoms, may come to be based. What is required merely is that those physicians having access to clinical cases should perform lumbar puncture early[2] when poliomyelitis is suspected, and during the prevalence of an epidemic on patients presenting a series of indefinite symptoms that precede as prodromata the onset of paralysis, or themselves comprise the group-symptoms that may define the so-called abortive form of the affection.

IMMUNITY

Experiments have been conducted to determine the kinds and degrees of immunity which are produced by the inoculation of the virus of poliomyelitis. Since the literature on epidemic poliomyelitis is silent on the subject of reinfection, it can be inferred that two attacks of the disease are rarely if ever suffered by one individual. Two possible reasons can be assigned for this: The first and most probable is that one attack of this disease, as is the case with some other acute general infections, tends to afford an enduring immunity, and the other, that as epidemics have in the past occurred

2. The earliest cellular reaction in monkeys is not purely lymphocytic but includes polymorphonuclear leukocytes which may predominate for the first period of one or two days. The earliest reaction of the meninges in human beings also is attended with polymorphonuclear excess (Wickman and Fulton).

infrequently and reappeared after long intervals, the children once affected have passed beyond the susceptible age period at the time of the next epidemic.

We have available in monkeys which have recovered from an undoubted infection, attended by paralysis, an opportunity to decide the question whether an attack of poliomyelitis protects against subsequent infection. Thus far we have subjected a number of monkeys to reinoculation into the brain at periods varying from eight days to four or five months, after the paralysis first appeared, without succeeding in reinfecting any of these animals. They were not rendered perceptibly sick by the subsequent inoculation after the one originally producing paralysis, and in no instance was recrudescence of the paralysis produced. It is interesting to note that in point of severity the first attack varied between mere tremor of the head, a partial paralysis of one limb, and complete paralysis of legs and arms. The paralysis had in some instances nearly or completely disappeared, and in others it had become reduced, but still affected all the muscles of one or two limbs. These results indicate what will probably be found to be true equally of human beings, that an undoubted attack of poliomyelitis, even when unaccompanied by definite paralysis, produces a state of refractoriness to reinoculation with active virus that endures for months and probably for years, if not throughout the life of the affected individual. On the other hand, our experiments, embracing now a large number of monkeys, establish that when the virus is introduced into the brain, practically all monkeys belonging to the generally susceptible species are subject to infection, although they do not invariably succumb to the first inoculation, but may require a second injection. However, it would still appear that rare individual monkeys are highly refractory to infection, and it has also been observed that an unsuccessful inoculation of the virus does not act as a protective, but leaves the unaffected animals either with their original degree of susceptibility unimpaired, or, what further experimentation will be needed to confirm, leaves the animals possibly somewhat more disposed to a subsequent infection.

This latter point of the failure of an unsuccessful intracerebral inoculation of the virus to increase resistance does not answer the question negatively, whether

by suitable means it may not be possible to develop an
active immunity independently of the production of even
the most trifling symptoms that might be taken to indi-
cate an attack of poliomyelitis. We have, indeed, been
successful in establishing in a certain number of mon-
keys a state of active immunity through a single large
subcutaneous injection of the crude or modified virus,
as represented by the emulsions of the spinal cord taken
from recently paralyzed monkeys, or by repeated injec-
tions of gradually increased amounts of the crude virus.
This mode of producing active immunity has not, up
to the present, been developed into a uniformly success-
ful and safe method, since of the treated animals some
do not develop a strong immunity and others develop
paralysis as a result of the treatment. The direct
immunizing effect has been produced also by means of a
virus somewhat modified after a long immersion in gly-
cerin, so that it may be regarded as probable that a quali-
tatively modified virus, changed through the action of
chemical or physical agents, or modified by biological
conditions within certain hosts, can be produced, and
that this altered virus will be found more appropriate
than the virus of full potency for purposes of active
immunization. At present the experimental basis is
entirely inadequate to justify the attempt to induce
active immunity as a protective measure in human
beings. Monkeys thus directly immunized resist suc-
cessfully the injection of large doses of virus into the
brain.

It has therefore been established that an undoubted
and even high degree of immunity to infection with the
virus of poliomyelitis is obtainable in animals, and
probably equally in human beings. The question which
next arises is this: As a result of this immunity, do the
body fluids acquire new properties capable of experi-
mental demonstration, on which the immunity probably
depends? In answer it can be stated that human beings
and monkeys who have passed through an attack of pol-
iomyelitis come to contain in their blood certain neu-
tralizing principles for the virus of poliomyelitis, and
that these principles are readily demonstrable by animal
tests for two or more years in human beings, and that
they probably persist for as great a period in monkeys.
Similar principles are absent from the normal serum.

They are demonstrated by bringing into contact an
active filtrate containing the virus with the serum of
the blood derived from animals or human beings who
have recovered from poliomyelitis, or from monkeys
actively immunized directly to the virus, and incubating
the mixture for a period at 37 C. and determining that
after this treatment the virus is no longer able to set up
paralysis on being injected into the brain of normal
monkeys. Furthermore, it has been shown by Netter
and Levaditi that the blood of a patient who had suf-
fered an abortive attack of poliomyelitis contained such
neutralizing principles.

A pressing question could now be approached, namely,
whether the immunity principles contained within the
serum suffice to neutralize the virus of poliomyelitis
once it is present in the body. It could be assumed that
the serum of animals, as of human beings that had
recovered from paralysis, would contain the immunity
principles in no high state of concentration. Effort was
therefore made to reinforce the degree of immunity in
these monkeys by subsequent inoculations of virus, and
the therapeutic experiments defined were conducted in
part with this reinforced serum, and in part with the
serum of directly immunized monkeys, and such serum
as could be obtained from human patients. It can now
be stated that if the quantity of the virus injected into
the brain be not in excess of a given dose the develop-
ment of paralysis can in some cases be prevented by mak-
ing several injections of the serum by lumbar puncture
into the subarachnoid space, while in another number of
animals the onset of paralysis is much delayed. In
respect to the latter point it may be stated that the
period of incubation in the control animals ranged from
seven to eleven days, and in some of the animals treated
with the human serum the onset of paralysis was delayed
until the twenty-sixth or twenty-seventh day. More-
over, infection by the nasal mucosa can also and with
greater certainty be prevented by the serum injections.

The manner of making the therapeutic injections was
somewhat varied, but in general may be described as
follows: An intracerebral injection of the virus having
been made, after the expiration of a period not exceeding
twenty-four hours, the subarachnoid injections of the
serum were performed on three successive days, after

which, an interval of three days having been allowed to
elapse, three more daily injections of serum were given.
We chose the subarachnoid method of injection rather
than the intravenous mode, in order that the immunity
principles might be brought into the most direct rela-
tionship with the virus, and particularly in view of our
conception of the pathogenesis of poliomyelitis and of
the primary site of localization of the virus in the lepto-
meninges. We believe that as good results could not be
achieved by the intravenous injection of the serum,
although the immunity principles could doubtless be
brought to the nervous tissues by the circulating blood,
for the reason that we know that these principles are not
readily if at all secreted into the subarachnoid space.
Hence they would not come into relationship with the
seat of the active virus at a period preceding its active
proliferation and extension throughout the nervous tis-
sues. We think it also probable that had the serum
injections been resumed after another interval of rest,
the last vestiges of the virus in the tissues might have
been destroyed in those animals in which the incubation
period was much delayed, so that the ultimate appear-
ance of paralysis might have been entirely prevented.

We have sought to ascertain whether animals protected
from infection through the employment of mixtures of
virus and immune sera, or as a result of treatment by
subarachnoid injections of immune sera for therapeutic
purposes, exhibited an unusual degree of resistance to
subsequent intracerebral injections of active virus made
at periods of several weeks to four or five months after
the conclusion of the original experiments. The object
of these tests was to ascertain whether a permanent
augmentation of the natural resistance to infection had
been accomplished by the introduction of the virus under
these conditions of restraint into the body. We have
found that no unusual degree of resistance to subsequent
intracerebral injections of active virus has resulted,
which fact we believe points to the validity of the fol-
lowing deductions: (1) A neutralized mixture of virus
and immune serum does not lead to any degree of active
immunization; (2) the therapeutic action of an immune
serum is associated with restraint of multiplication of
the virus such as would be required to establish any

grade of active immunity; (3) a simple passive immun-
ity is either not produced at all by the serum injections,
or is of brief duration or small amount.

PRODUCTION OF A SERUM

The observations on immunity which have been pre-
sented early suggested that an effort be made to produce
a corresponding active immunity in some of the lower
animals that might possibly become the source of a
therapeutic serum. This particular phase of our studies
is being actively pursued at the present time. However,
several facts have already been developed. It has, for
instance, been found that the horse does not readily
respond even to large injections of filtrates carrying the
active virus with the development of immunity princi-
ples within the blood. It is true that our experiments
are restricted for the present to a single horse, the blood
serum of which, after many months of treatment, had
no restraining effect on the virus either *in vitro* or within
the body. The rabbit and chicken would seem not to
yield such immunity principles. On the other hand we
have secured indications that the sheep may react more
favorably. It has been found that normal sheep serum
possesses a definite although slight neutralizing power
for the filtered virus when mixed directly with it, and
that the injection of emulsions of the spinal cord and
brain of recently paralyzed monkeys into the sheep aug-
ments this property of neutralization. What remains to
be determined is the degree to which this augmentation
can be carried, and whether a serum can be secured that
will possess therapeutic value; but should the sheep fail
in this respect, the search must be continued and be
made to include still other species of animals, in the
hope that one will be found in which the immunity
response will be greater. There are serious objections
to the use of monkeys as a source of a therapeutic
serum, even were one of high potency capable of being
prepared in those animals. The use of human serum,
derived from persons who have passed through an attack
of poliomyelitis, as a therapeutic agent, would be free
of certain objections attached to the use of monkey
serum, but it is not very probable that such an immune
serum would be found to have sufficient strength or that
it could be procured with sufficient readiness to make it

available for practical application. Finally, the serum treatment of poliomyelitis must at the present time be regarded as strictly in the experimental state, and it cannot be predicted how soon or whether ever at all such a form of specific treatment of the disease will be applicable to the spontaneous epidemic disease in human beings.

SUMMARY

It may be confidently stated that the experimental study of poliomyelitis has yielded a large number of important facts relating to the spontaneous disease in man. These facts include the discovery of the nature of the virus, of many of its properties, of certain important clinical and pathological peculiarities of the disease, of the phenomena of immunity, of a mode of spontaneous infection, while they have served to establish a basis on which to develop measures of prevention, and on which to build hopes for the working out of a specific method of treatment. Moreover, the advance which has now been accomplished will make it possible to determine with accuracy whether poliomyelitis is a single clinical and pathologic entity, or a generic name covering groups of symptoms and descriptive of the effects of certain lesions of the spinal cord and brain of which epidemic poliomyelitis is merely one, but the most important variety. It is not improbable that poliomyelitis, like meningitis, may be found to arise from several independent causes, and that epidemic poliomyelitis, like epidemic meningitis, may be distinguished among the varieties by being invariably the product of a specific micro-organism. We possess already evidence to the effect that certain of the lower animals, among them poultry, the dog, and possibly the horse, are subject to poliomyelitis, due apparently in each instance to a cause peculiar to the species affected.

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Sixty-sixth Street and Avenue A.

The Treatment of Syphilis with Ehrlich's "606"

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PART I. THE THEORETICAL AND EXPERIMENTAL BASIS FOR THE USE OF "606"

The object of experimental chemotherapy is the preparation and discovery of drugs which shall possess specific powers over given parasitic micro-organisms. The pursuit of this object has recently been greatly promoted by the discovery by Professor Ehrlich of a drug which possesses extraordinary power of destruction over certain, at least, of the parasitic spirochetes. Ehrlich has for many years been engaged in the synthesis of drugs, the ultimate purpose of which was the destruction within the body of such parasitic organisms as trypanosomes and spirochetes under conditions in which the organic cells of the host would be left unaffected. In other words, and to use Ehrlich's terminology, it was necessary to seek drugs which were on the one hand highly parasitotropic and on the other were quite devoid of organotropic activities. In the course of this quest, Ehrlich has had the good fortune to produce a number of drugs which more or less fulfilled these ideal conditions, but recently the quest has been crowned with an extraordinary achievement that promises to be of the greatest importance in the treatment of syphilis and hence of incalculable benefit to the human race.

We may begin by summarizing a fragment of the conceptions which led Ehrlich to his most recent important

discovery: The animal parasites causing malaria, try-panosomiasis, syphilis, amebic dysentery, etc., apparently can not be successfully attacked by means of immune sera, and therefore their conquest must be attempted by means of ordinary chemical substances. The history of the treatment of malaria with quinin indicated that this line of attack is a feasible one, particularly in view of the fact that quinin represents the successful employment of one drug out of an innumerable series tried by man in nature. It is possible through the aid of modern synthetic chemistry to prepare an almost infinite number of combinations which can then be tested on animals infected with the animal parasites of the diseases named, until compounds are found which exhibit a higher degree of poisonous effect on the parasite than on the host. Having achieved this first selection, the partially successful compound can then be further altered by possible substitutions, which may serve to render it still more suitable for the purposes in view.

While Ehrlich was engaged in developing this line of investigation, the substance atoxyl, which is an empirical arsenical compound, came into use in the treatment of sleeping sickness, for which it proved a highly useful although not invariably successful drug. This substance, which was demonstrated to be more poisonous for one of these animal parasites than for the human host, became the starting point for chemical variations which culminated in the preparation of the compound "606." The starting point of the successful substitutions in connection with atoxyl was the working out of its real constitution. It had been considered as an anilid of arsenic acid,

NH. As O$_2$
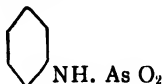
which, being an unstable compound, would not allow of the necessary chemical manipulation and substitution. In collaboration with his chemical assistant, Bertheim, Ehrlich ascertained that atoxyl is in fact the sodium salt of para-aminophenyl arsenic acid,

NH$_2$

Na O-As=O
|
OH
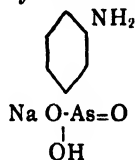
In this compound the molecule of arsenic is firmly attached to the benzole ring, because of which the compound admits of the necessary manipulation and substitution at different positions in the ring. Different substitution products were next prepared and tested for their therapeutic effects on infected animals. Up to the present time, about 630 substitution products have been made and tested, of which four only have proved to possess the requisite parasitotropic properties, without at the same time being injurious to the organs of the host. These substances are: acetyl-atoxyl; arsenophenylglycin, or "418," tryparosan, and arsenobenzol, or "606." Excepting the drug tryparosan, arsenic is the active principle in all the preparations, the other chemical groups present merely serving to fix this substance to the parasite. Thus for example it has been found that the acetyl group has a special affinity for trypanosomes and the amino and hydroxyl groups a special affinity for the spirochetes.

It is obvious that this kind of pharmacologic research is possible only if animals can be used as the basis of experiment. Fortunately, the discovery of the transmissibility of syphilis to the lower animals and of *Treponema pallidum*, the parasitic cause of syphilis, came to be achieved about this time. Hence the new drugs could be tested not only against fowl spirilla and the spiral organisms of relapsing fever, but also against *Treponema pallidum* in infected rabbits. In due time preparation No. "606" came thus to be tested, and was proved to be efficient in a single dose, which while it sufficed to destroy all the parasites was without perceptible injurious effect on the host.

In the course of previous investigations, Ehrlich had ascertained that it was not safe to attempt to destroy the animal parasites by means of repeated small doses of an injurious drug, and for the reason that in the course of this method of treatment, the parasites often develop a toleration for the drug which they transmit to their progeny. The goal, therefore, became the discovery of a drug which in a single dose would destroy all the parasites, leaving the host uninjured, and this extraordinary achievement apparently is accomplished by "606."

The drug having been proved by repeated tests on animals to be without injurious effect it was next tested on human beings. The first to submit to inoculation were the assistants, since which time several thousand persons suffering from various forms and consequences of syphilis have been treated.

The reports which are appearing at present in practically every number of the important German medical weeklies from different parts of Germany and Austria are almost unanimous in their testimony to the remarkable effects of the drug in syphilis. In all but a few exceptional instances a single dose of the drug has sufficed to bring about a rapid disappearance of the lesions of syphilis and equally rapid improvement in the general condition of the patients. In the few instances in which these rapid effects were not achieved the dose of the drug, which was originally given was, as is now established, too small, and in many of these cases a second and larger injection has brought about the desired result. It is noteworthy that in many instances the patients who have improved rapidly after an injection of this drug had resisted the application of mercury sometimes over many months, or possessed idiosyncracies which made the use of mercury difficult or impossible. Thus far, a special idiosyncracy against the drug has not come under notice. It is as yet too early to determine whether the patients who have responded so rapidly and perfectly to the administration of the drug as apparently to have been cured of this otherwise chronic disease by a single injection may ultimately suffer relapses. On the other hand many scores of patients have shown no relapses in the several months which have elapsed since the injections. What is highly important in the interests of prophylaxis is that within twenty-four or forty-eight hours of the injection of the drug superficial lesions, such as mucous patches, condylomata and primary lesions are freed from living spirochetes. This remarkable effect of the drug on the parasites can be readily demonstrated on the testicular spirochetal lesions of the rabbit, in which the innumerable spirochetes can be entirely immobilized within twenty-four hours with a single injection of the drug, after which the lesion quickly resolves.

ADMINISTRATION OF THE DRUG

The chemical name of "606" is paradiamidodioxy-arsenobenzole dihydrochlorid.

BASE   ALKALINE SALT

As=As   As=As

H_2N NH_2 H_2N NH_2

OH   OH   ONa   ONa

ACID SALT

As As

$ClH.H_2N$ $NH_2.HCl$

OH   OH
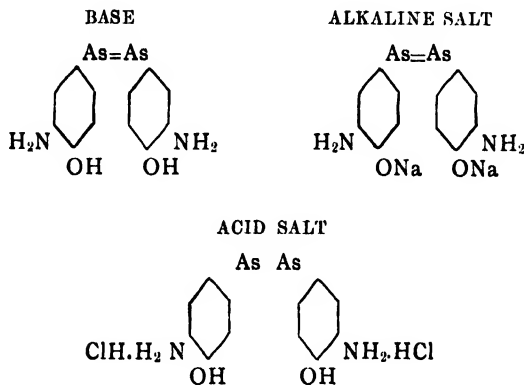
The substance is a yellowish powder which rapidly oxydizes on exposure to air, and is therefore put up in vacuum tubes. It dissolves in water with difficulty, making a strongly acid solution. As the acid solution is very painful, the substance is administered either as a neutral base (Wechselmann) or as an alkaline salt (Alt). The administration is by injection deep into the muscles, or into the veins or beneath the skin. At first, the administration was made either deeply into the muscles of the buttocks or into the circulation, but at present it is recommended that the administration be made subcutaneously according to the method of Wechselmann.

According to this method, the drug, in a dose which has varied up to the present from 0.3 to 0.6 grams, is dissolved in a mortar in 1 to 2 c.c. of ordinary solution of sodium hydrate. Acetic acid is then added, drop by drop, until the base precipitates out in the form of a fine yellowish suspension. This precipitate is collected in from 1 to 2 c.c. of sterile distilled water, and there are added either 1/10 normal sodium hydrate, or 1 per cent. acetic acid, as needed, until the reaction becomes precisely neutral to litmus. According as the reaction is or is not accurately neutral the injection will be followed by much, little or no pain. It is moreover desirable to subdivide the precipitate as finely as possible, which can be done by rubbing. The suspension is then drawn into a suitable syringe and injected subcutaneously below the

No.	Age.	Sex.	Infection.	Previous Treatment.	Condition Before Injection.	Injection.
1	25	M	1/10, chancre; 2/10, secondaries	11 injections 1 gr. Hg Sal.	General secondary eruption; enlarged lymph nodes; serum+	5/12/'10; 8 c.c. each buttock. 0.3 606, not completely dissolved. Alt method.
2	38	M	1905, chancre and secondaries.	Irregular.....	Tertiary syphiloma of back and chest; serum + +	5/12/'10; 8 c.c. each buttock. 0.3 gm. 606, not completely dissolved. Alt method.
3	21	M	3/1910, chancre; 5/1910, secondaries.	Only local treatment.	Circinate syphilid of face; macular syphilid of trunk; enlarged lymph nodes; serum ++	5/16/'10; 0.3 gm. 606; 8 c.c. each buttock. Alt method.
4	32	M	5/1907, chancre and secondaries.	More or less constant; liquid.	Scaling syphilid of palms severe. Serum ++	5/16/'10; 0.3 gm.; 12 c.c. eac buttock. Alt method.
5	20	F	4/1910, secondaries.	4 grs. Hg Sal. by injection	6 mos. regnant; faint eruption. Serum +	5/19; 0.3 gm. 606; 10 c.c. each buttock. Methyl alcohol.
6	24	M	10/1909, chancre; 12/1909, secondaries	Mercury in pill form for several months.	Severe rupial lesions, face, head and arms; hysterical. Serum ++	5/19; 0.3 gm. 606; 10 c.c. each buttock. Methyl alcohol.
7	54	M	1882,. chancre and secondaries.	10 days treatment in 1882.	Ulcerating syphilid of knee and leg. Serum .++	5/23; 0.3 gm. 606; 10 c c. each buttock. Methyl alcohol.
8	25	M	10/1909, chancre; 1/1910, secondaries	2 grs. Hg Sal. by injection	Mucous patches, fading follicular syphilids of body. Serum +	5/23; .03 gm. 606; 10 c.c. each buttock. Methyl alcohol.
9	28	M	5/1909, chancre; 9/1909, secondaries	"1 bottle of medicine."	Severe extensive pustular and tubercular syphilids of face and shoulders. Serum ++	5/26; 0.3 gm. 606; 10 c.c. each buttock. Methyl alcohol.
10	24	M	1907, chancre and secondaries.	KI 7 months	Severe periostitis, left tibia. Serum +	6/2; 0.3 gm. 606; 10 c.c. each buttock. Methyl alcohol.
11	28	M	1/1910, chancre; 5/1910, secondaries	No treatm't.	Mucous patches and macular eruption on face and legs. Serum +	6/2; 0.3 gm. 606; 10 c.c. each buttock. Methyl alcohol.
12	22	M	4/1910, 3 chancres on lips; 5/1910, secondary eruption.	2 grs. Hg Sal. hypodermically.	3 large indurated chancres on lips; erythematous papular eruption on body. Serum ++	6/2; 0.3 gm. 606; 10 c.c. each buttock. Methyl alcohol.
13	29	M	1905, chancre and secondaries.	Treated 4 wks. in hospital, 1905; 1906, 8 weeks in hospital.	Arthritis of knee, broken down glands of groin, emaciated, anemic. Serum +	6/7; .03 gm. 606; 7 c.c. each buttock Methyl alcohol.
14	20	F	6/1910, chancre and secondaries.	No treatment.	Mucous patches, general eruption. Serum.	6/7; 0.3 gm. 606; 7 c.c. each buttock. Methyl alcohol.

Local Effects.	Condition After Injection.	Serum Reaction.	Remarks.
Induration for 7 days, slight pain.	No improvement in 3 weeks.	6/16/'10+35 days.	Small dose, resumed mercury.
Marked induration, 7 das. scaly erythema of hands and feet.	Slight improvement in 3 weeks.	6/10/'10++29 das.	Small dose, resumed mercury and KI; drug eruption.
Induration of buttocks for 7 days.	After 8 dys. lesions gone on face, and faint on body.	5/23/'10 + 7 days.	Left hospital 5, 24, '10, considering himself well. Could not be traced.
Induration lasting 7 das., slight fever 2 days.	50% improvement in palms after 7 days. Completely healed in 1 month.	6/10/'10—25 days.	Began to work as orderly in hosp. 6/6; apparently cured by single injection. See Photographs Figs. 1 and 2.
Marked induration 5 days, urticarial eruption 2 days.	Apparently well in 21 days.	6/28 — 39 days....	Aborted 5/29; fetus badly macerated, could not be obtained for examination.
Slight induration 4 days.	Marked rapid and progressive improvement. Lesions healed in 39 days.	8/30 — 3 mos. 11 days.	Gained 12 pounds. No recurrence in 3 mos. Photograph Figs. 3, 4.
Marked induration of buttocks 7 days.	Sinuses closed induration gone and lesions of light color 21 days.	6/10 — 21 days....	Apparently cured.
Slight induration.	Marked improvement, lesions gone in 2 weeks.	6/11 — 19 days....	Apparently cured by single injection.
Induration of buttock 5 das.	Marked improvement. Lesions dry and faint in 14 days.	....	Gain in weight. Left hospital and could not be traced.
Marked induration 7 days.	Pain and swelling disappeared in 8 days.	8/30 — 3 mos.	No recurrence in 3 mos.
Induration 7 days.	Face and leg clear in 7 days.	6/23 — 21 days....	Apparently cured by single injection.
Induration of buttock 5 days.	Chancre gone, eruption very faint; 21 days.	....	Great improvement in general condition. Photograph Figs. 5, 6. Could not be traced after leaving hospital.
Slight induration.	Marked and rapid improvement in general condition and lesions in 10 days.	6/30 — 23 days....	Apparently cured; 8/30 working as longshoreman; has gained 30 lbs.; serum —
Induration 5 days.	Eruption and sores disappeared in 10 days.	....	Left hospital and could not be traced.

shoulder blades after previous cleansing and disinfection
of the part. It often happens that there is slight pain
lasting a few minutes following the injection, and in
some instances a slight swelling arises on the second or
third day following the injection, but no bad effects are
produced. There may be slight rise of temperature and
in some instances an urticarial eruption has occurred,
but no specific toxic effects on the eyes, kidneys, or nerv-
ous system have been observed.

PERSONAL OBSERVATIONS

Although the number of cases to be reported on at
present is small, the results have been so striking, and
are in such accurate accord with the several thousands
of cases reported from Germany that it has seemed desir-
able to make this report, especially because it is probable
that the drug will come to be extensively used throughout
the United States. The patients were all in the City
Hospital on Blackwell's Island, in the skin and venereal
wards. The clinical diagnoses were all definite and un-
mistakable, and the serum reactions which were made
regularly were conducted with the Noguchi modification
of the Wassermann method.

In injecting the preparation into the first two patients
considerable difficulty was experienced in getting the
powder into solution, and the full dose of 0.3 gram which
it was intended to administer was therefore not given.
As we now know, 0.3 gram is a submaximal dose. These
patients were injected before the method of Wechsel-
mann had come into use, and even before the earlier
modification of the original "Alt" method of preparation
suggested by Ehrlich had been published. As a result
no marked improvement occurred and the patients were
put back on mercurial treatment. The remaining twelve
received the full dose intended, and included examples
of primary, secondary and tertiary lesions. The effects
were in these instances truly remarkable and now, from
three to four months after the injections relapses have
not been observed to occur. Before the treatment the
serum reactions were all positive and at the expiration
of the time mentioned, in all the patients who could be
traced, they have become negative. Aside from the tem-
porary local disturbance caused by the injection in some
instances, no untoward symptoms were observed, except
an urticarial eruption in two of the cases. None of the

Fig. 1.—Scaling syphilid of palms. A. K. Duration of infection, three years; of palmar lesions, one year. May 16, 1910, 0.3 gm. 606. Wassermann before treatment, + + ; June 10, 1910, negative; Sept. 1, 1910, still negative. (See photograph after treatment.)

patients received more than 0.3 gram, which is about one-half of the dose now being employed abroad. Hence it would not be surprising if in this first group relapses occurred. If so it would be both safe and desirable that a second and larger injection be given.

The efficiency of the drug is dependent in part on the dose employed. Hata has permanently cured all rabbits having spirochetal lesions by means of a single dose of an adequate size. We have found that when the dose is too small, relapses occur in the infected rabbits. Hence

Fig. 2.—Scaling syphilid of palms. A. K. Condition of palms two weeks after treatment with 606. (See photograph before treatment.)

now that it has been determined that a dose of 0.6 gram is non-toxic for human beings, it is probable that the average dose will approach that quantity. At present, European observers are employing doses varying from 0.45 to 0.6 gram.

The drug is not generally available at the present time. The output is too small for general distribution, and Ehrlich has therefore up to the present been able to sup-

ply it to relatively few physicians, whose employment of
it has also been in the nature of tests of its value. The
final word concerning its value will not of course be said
for a number of years, but the fact remains that we pos-
sess no drug the extraordinary effects of which in syph-
ilis equals that of "606." The one drug with which it
may be compared is quinin, which in many instances is
so highly efficient in the treatment of malaria, but even
there a single dose does not suffice to destroy all imme-
diately visible parasites, as does an adequate dose of
"606."

Fig. 3.—Nodular and ulcerating syphilid. W. C. N., date of luetic
infection, October, 1909. Rupial and fungating lesions of face and
arms of six months' duration. May 19, 1910, received 0.3 gm. 606.
Wassermann before treatment, ++; Aug. 30, 1910, negative. (See
photograph after treatment.)

PART II. THE USE OF "606" FROM THE STANDPOINT OF THE CLINICIAN

Personal experience in the treatment of syphilis has
convinced us that mercury given in the early stages in
as large doses as the system will tolerate insures the
patient more certainly against relapses, and that patients

who are treated by small doses over long periods of time in the manner which is still too often employed by even the best known syphilographers sometimes develops in the patient thus treated an immunity to the drug which renders it comparatively inert. This latter class of patients not infrequently become the subjects of obstinate relapsing lesions of the skin and mucous membrane or, later, the victims of paresis, tabes, or other visceral or bone affections. Professor Ehrlich's contention, there-
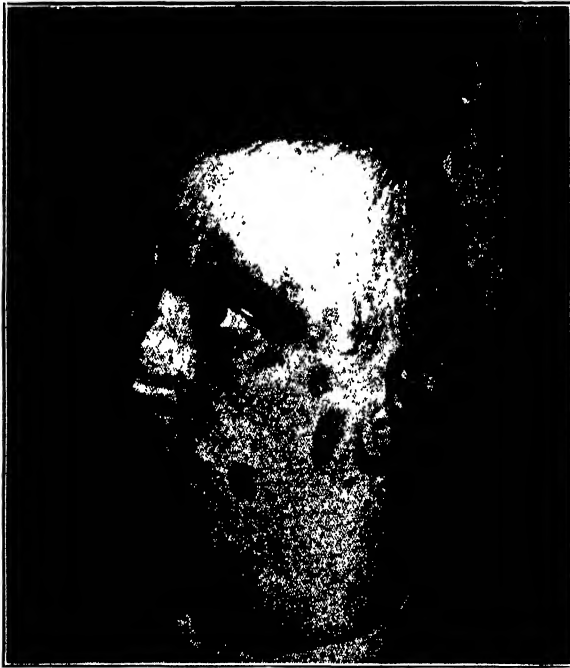
Fig. 4.—Nodular and ulcerating syphilid. W. C. N. After treatment with 606. (See photograph before treatment.)

fore, as to the value of a drug which at one dose destroys the invading organism is supported by clinical experience in the use of mercury. It is true that the methods of administering the latter have been greatly improved in the last few years, as is witnessed by the greater frequency with which it is employed hypodermically and by inunction, but our present means are still too often slow or inefficient in their action especially in the early stages. Delay or insufficiency in the amount of mer-

cury at this time permits the entire system to become
infected by the *Treponema pallidum* and damage to the
blood-vessels or important structures takes place which
might be prevented by energetic treatment. It will gen-
erally be conceded by physicians who come in contact
with a large number of syphilitics that while mercury
and potassium iodid are efficient in the great majority
of cases in controlling the manifestations of the disease,
there are certain intractable cases in which they fail to

Fig. 5.—Multiple chancres. J. S. C., infection two months ago.
Two chancres on upper lip and one on lower in median line:
papulo-erythematous eruption on body. June 2, 1910, 0.3 gm. 606.
Wassermann before treatment, + +. (See photograph after treat-
ment.)

control the symptoms either on account of idiosyncrasy
to one or both of these drugs or other causes. Further-
more, owing to the length of time required in treating
a case of this infection patients often become discour-
aged and cease medication after a few months or a year
or two. The great advance made in controlling the

treatment by the Wassermann reaction cannot be too strongly emphasized, showing as it does that in the past some patients may have been greatly overtreated while the majority have received too little of the specific drugs. It is well known that there are certain cutaneous and general manifestations of syphilis which respond slowly or not at all to mercury even in heroic doses. Among these may be mentioned certain scaling syphilids of the palms, chronic nodular syphilids in patches of the
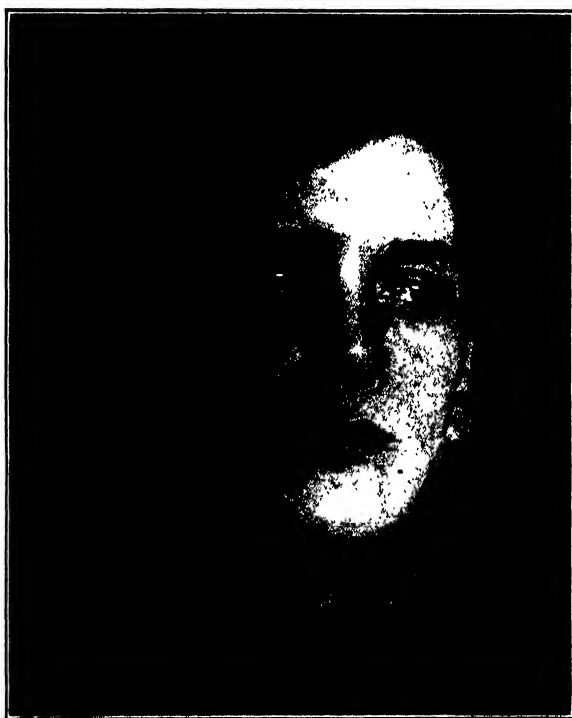
Fig. 6.—Multiple chancres. J. S. C. After treatment with 606. (See photograph before treatment.)

type which clinically resembles lupus vulgaris, certain forms which affect the flush area of the face and bear a marked clinical resemblance to lupus erythematosus; also mucous membrane affections like leucoplakia, chronic interstitial glossitis and relapsing deep and superficial lesions of the tongue and throat. Aside from these lesions which are under direct observation there are the so-called parasyphilitic manifestations, such as tabes,

the treatment of which by the classical drugs leaves much to be desired. In malignant syphilis with early destructive lesions and the development of profound cachexia, mercury, moreover, often fails to do good and not infrequently does harm. When, therefore, Dr. Flexner, of the Rockefeller Institute, in the early part of May, this year, received a supply of the new preparation "606" and asked to be allowed to try it in a number of selected cases in the City Hospital, the proposition was received with favor and the patients in the skin and venereal wards were placed at his disposal. At that time the experience in the use of the drug had been rather limited and the patients who were treated were carefully selected as free from organic disease of the kidneys, heart or eyes; in fact, all the contraindications for the use of the drug, as stated by Prof. Ehrlich, were carefully observed. The patients were also informed that a new drug was about to be employed which would cause them a considerable amount of pain and was yet in the experimental stage. There was no difficulty in obtaining their consent, and the most striking testimony as to its value in the treatment of obstinate cases of lues was furnished by other patients in the wards, who on seeing the results accomplished by its use repeatedly asked that it be given to them.

The rapid action of arsenobenzol in cases of obstinate syphilis of the palms is strikingly illustrated in Figures 1 and 2.

Patient, aged 32, had a genital chancre three years before, which was followed by the usual secondary symptoms. Following his infection he had taken medicine in "liquid and capsule" form almost continuously. The eruption on his hands was of one year's duration. The Wassermann reaction was strongly positive. On May 16, 1910, he received 0.3 gm. "606." Two weeks afterward his palms presented the appearance shown in Figure 2 and in another fortnight he was entirely well.

The result obtained by the use of "606" in this case was far more rapid than any which we have seen after the use of mercury. Furthermore, after an apparent cure from mercury in these cases of chronic palmar syphilis the majority relapse within a few weeks to a month or two, whereas this patient had had no recurrence up to September first and the Wassermann reaction has remained negative.

In the case of multiple initial lesions of the lip (Figures 5 and 6) of two months' duration, with secondary manifestations of the skin, complete resolution of the chancres took place within about ten days, leaving practically no evidence of their former existence. The entire appearance of the patient, too, underwent a marked change, as before treatment he was anemic, depressed, and evidently showing considerable constitutional disturbance from his infection. The great improvement which took place in his general condition after the injection continued up to the time he left the hospital. The Wassermann reaction at that time was negative. The duration of the initial sclerosis under mercurial treatment is a very variable one. It is unusual, however, to obtain complete resolution under several weeks to two or three months; in fact, the induration sometimes persists for a year or longer. As these extragenital initial lesions are a source of great mental distress to the patient and one of danger to his environment, their rapid retrogression under this remedy surpassed the result which could have been expected from mercury.

Figures 3 and 4 illustrate a rather malignant form of eruption which occurred less than one year after infection:

Patient, aged 24, contracted syphilis in October, 1909. A month later a pustular eruption appeared first on his face and then on the body. The lesions become rupial and afterward assumed a fungating type. He also suffered from sore throat, headaches and osteocopic pains. From October up to the time he entered the hospital he had taken mercury in pill form. On May 19, 1910, he received 0.3 gm. "606." There was a perceptible change after a week; the patient's general condition improved and in two weeks he gained 9 pounds.

This form of early malignant syphilis, which indicates low resisting power on the part of the infected individual, sometimes fails to respond to mercury. The almost magical result which followed the use of "606" is illustrated in Figure 4 which was taken about two weeks after the administration of the drug. Up to September 1 the patient had remained well and showed a negative Wassermann reaction.

The cases just referred to are examples of the most brilliant results obtained. In the table additional data are given bearing on the other cases which were treated

at the City Hospital. Through the kindness of Dr. Flexner, we have been able in the past month to secure a sufficient quantity of the remedy to treat several private patients who are still under observation; the cases will be reported in greater detail later. One of these was a case of rapidly progressing optic neuritis with tabetic symptoms, the result of a specific infection twenty years before:

Patient, referred by Dr. Marks of Frankfort, was a man, aged 38, in whom it was noticed two and a half years ago that his pupils were smaller than normal. Shortly afterward, he developed a diplopia which was diagnosed by the ophthalmologist as paralysis of the right abducens and beginning atrophy of the optic nerve. From that time until August 1 he had been under active mercurial treatment but without benefit. His vision since April, 1910, has been rapidly diminishing and he has concentrically contracted field, Argyll-Robertson pupil and partial atrophy of both optic nerves. Although we recognized that affections of the optic nerve are given as a theoretical contraindication to the use of "606," still at the urgent solicitation of the patient and his friends the drug was administered, but he was told beforehand that very little result was to be hoped for. A dose, 0.45 gm., was prepared after the method of Wechselmann and injected beneath the right shoulder blade. This was followed by severe pain which persisted for three or four days and had to be controlled by several hypodermics of morphin. On the second day, a marked swelling appeared at the site of injection which began to resolve at the end of a week. At no time was there any evidence of softening. The patient's temperature rose slightly on the second day reaching 100.4 F.; his pulse was rather high for the first week varying from 90 to 110 and he developed considerable physical weakness. Although little result was expected from the drug in this case, the patient is under the impression that the rapid progress of the disease has been arrested.

In the following case of cerebral syphilis an improvement took place in some of the symptoms which mercury and other medication had failed to influence:

Patient was a man, aged 38, whose infection dated back six years. In February, 1910, the neurologic examination made by Dr. M. G. Schlapp was as follows: "Pupils unequal, the right being larger; they react to light, not to accommodation—very nearly Argyll-Robertson condition. Speech thick, not truly scanning. General involvement of the muscles of the pharnyx, tongue and face on right side; anesthesia in the same area. Partial paralysis in the right leg. Foot

dragged in walking; weakness in the leg is sufficient to prevent standing on it. Babinski sign pronounced; clonus in both knee and ankle, the whole leg anesthetic." In spite of vigorous mercurial and iodin medication there occurred an increase in symptoms and area involved in June, 1910, while staying in England. The patient lost consciousness on several occasions; he also had facial paralysis complete for a time on the right side. Both legs developed weakness and anesthesia (patient's statement). A similar attack occurred in July, 1910, after a slight improvement, again involving both legs and right side of the face. Incontinence of urine marked. Under 43 injections of a mercury arsenalate, 20 inunctions and a daily dose of 3 grams of sajodin, rest and hydrotherapy, sufficient improvement occurred to enable the patient to get about.

On August 31 he was given an injection of 0.45 gm. "606" subcutaneously. A Wassermann reaction on this day was positive. Absolutely no pain followed this injection, nor were any unpleasant effects of any kind experienced except the induration which persisted for ten days. Improvement was noted on the day following the injection and he left the hospital on the second day, remaining in bed, however, in his home for two or three days longer. On the day succeeding the injection the patient stated that the mental depression from which he suffered weeks before had become materially less, his facial paralysis showed improvement and his speech disturbance was less marked.

A second examination made by Dr. Schlapp on September 9, nine days after the injection of "606". is as follows: "Pupils in same condition as at first examination. Incontinence has ceased. Paralysis of face and speech muscles has improved; anesthesia the same as at first examination. Right-sided sweating of the face, neck and upper part of the trunk. Both legs show Babinski sign, ankle and knee clonus, indicating extension of the process since February. There is, however, a noticeable decrease in anesthesia and muscular weakness. Diagnosis: Syphilitic obliterating endarteritis of the base, involving both pyramidal tracts and the sympathetic."

The patient was kindly referred by Dr. James C. Johnston, under whose care he is at present. A further report will be made at a later date. (September 21, Wassermann reaction still + —.) The absence of pain in this case was probably due to greater experience and care in preparing the remedy. Iit is important to emphasizs the necessity of thoroughly rubbing the precipitate which follows the addition of acetic acid to the caustic soda solution until a homogeneous paste is obtained. The neutralization of the mixture should

then follow and the latest technic as given by Wechsel-mann[1] observed.

The question of danger in the use of the new drug is one that should be touched on. It would be a marvel should a drug so potent as this one in the destruction of living parasites within the body be wholly devoid of injurious or secondary effects on the organs. We possess indeed no really pharmacologically active drug for which there may not exist contraindications and from which secondary and objectionable effects have not been noted. Certainly neither mercury nor iodid of potassium form exceptions to this rule. The remarkable fact about "606" is that in the several thousand cases in which it has already been employed, and considering the wretched physical state in which many of the patients were at the time of the injection, so very few untoward effects have occurred. It is now clear that the mode of administration is important, and especially the elimination of the methyl alcohol used at an early period to dissolve the drug, since this substance may produce unpleasant if temporary symptoms, as in the three cases reported by Bohac and Sobotka.[2] The result in a case of Hoffmann's[3] in which a central embolic pneumonia, terminating in recovery, followed an intragluteal injection, was probably analogous to the condition of the lungs which may follow from an intramuscular injection of insoluble mercurial salts when the needle has inadvertently penetrated a vein. Ehrlich has warned against the administration of the drug to weak patients suffering from advanced cardiovascular disease, and the wisdom of this precaution has been emphasized by the two accidents encountered at the Charité in Berlin and the neurologic clinic at Bonn. Ehrlich further warns against the use of the drug in cases in which lesions of the optic nerve exist, and urges that in doubtful cases an ophthalmologic examination be made. Finally, it has just been announced that a further improvement in the preparation of "606" has been made which still further reduces the toxicity—indeed it is stated to be one-third that of the older substance. To this preparation Ehrlich has given the name "hyperideal."

1. New York Med. Jour., Sept. 3, 1910, p. 449.
2. Wien. klin. Wchnschr., July 28, 1910.
3. Med. Klin., Aug. 14, 1910, p. 1291.

From the collective reports of those who have used the drug the impression is gaining that we have in arsenobenzol a most thorough agent in controlling the manifestations of syphilis which are caused by the presence of the treponema. It may reasonably be hoped, therefore, that all the lesions which depend on the presence of the organism will be favorably influenced and the most we can expect in the secondary degenerative changes is that the process may become arrested. Further experience with the drug will determine with more accuracy the dose which is necessary to bring about a cure, the time that must elapse before a second dose can be safely given and the more definite indications for its use after relapses or failure of a single dose to control the symptoms.

The authors desire to acknowledge the courtesy of Dr. Robert H. Greene and Dr. Eugene Fuller in placing certain patients at their disposal.

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GRAFT OF THE VENA CAVA ON THE ABDOMINAL AORTA.

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I. INTRODUCTION.—The operation consisted of transplanting between the cut ends of the abdominal aorta a segment of vena cava. Its purpose was to study the details of the technic of the graft of a large vein on the aortic trunk and its remote results. It is important to know whether venous segments can replace parts of the aorta as successfully as parts of smaller arteries, the carotid for instance. Five years ago, I advocated the use of venous grafting in the treatment of aneurisms and of certain traumatic lesions of arteries.[1] It was warranted by the results of experiments that I had performed in 1902 in Lyons with Morel[2] and in 1905 in Chicago with Guthrie.[3] These experiments had shown that arteriovenous anastomoses made with a proper technic remain normal, and that a vein, transplanted on an artery, reacts against the increase of blood-pressure by thickening its wall. They demonstrated that veins can be safely grafted on arteries. At this time, it was believed that the successful arterial transplantation of a vein was not possible. Exner[4] had attempted several times to graft a segment of jugular vein on the carotid artery. The vein always became occluded. The results of Hoepfner[5] and of Goyanes[6] had also been negative. Payr[7] concluded that thrombosis was due to the action of the arterial blood-pressure on the thin venous wall, and that this biological factor rendered impossible the success of a venous transplantation. A short time afterwards, I was able to repeat these experiments at the University of Chicago. I found immediately that the thrombosis observed by previous experimenters was not due to a

biological but merely to a surgical cause, and that, with a proper technic, positive results could easily be obtained.   Our experiments of 1905 and 1906 showed that arteriovenous anastomosis remained normal after twenty months.   They also permitted the study of anatomical modifications of the venous wall under the influence of the increased blood-pressure. Eight months after the graft of a segment of jugular on the carotid, the circulation was found to be normal through the vein, the wall of which had become as resistant as an arterial wall.[8]   It was, therefore, certain that veins followed the law of adaptation of organ to function, and that they could, when they were compelled to do so, play the rôle of arteries.   In other experiments, made in 1907 and 1908 at the Rockefeller Institute, I was able to follow more closely the histological evolution of segments of jugular vein transplanted on the carotid, and observe that twenty months after the operation the venous segment was still in excellent condition.

These results have been confirmed by other experimenters in America and Europe.   In 1907, in the laboratory of Harvey Cushing at Johns Hopkins University, Watts [9] performed several times the graft of a segment of jugular vein on the carotid artery.   He found that the vein adapted itself to arterial function.   Stich,[10] during the experiments made in 1907 and 1908 in the Klinik of Garré, was able to follow the evolution of a venous segment grafted on a carotid artery for 409 days.   Fischer and Schmieden [11] also obtained positive results.   In 1909, at the Pasteur Institute, Frouin [12] performed some arteriovenous anastomoses which became occluded after a few months.   He did not realize that the occlusion was due to his defective technic, and concluded that it was the normal evolution of the anastomoses.

The information given by these experiments has been used only twice in human surgery.   Goyanes performed, in 1906, an incomplete transplantation of the popliteal vein on the popliteal artery after extirpation of an aneurism, and his patient recovered.   In 1907 in a case of axillary aneurism, Lexer [13] performed a graft of a segment of saphenous vein,

but his patient, whose general condition was very bad, died after a short time. On human beings, the technic of the arteriovenous anastomoses is easier than on dogs. They should be as successful, if they are performed with a proper technic and when the anatomical conditions of the artery allow it.

From an experimental stand-point the transplantation of venous segments can yield excellent results, even after a long time, when the operation is performed on dogs' carotid arteries. It is important to know whether these results will be different in case of larger and more friable vessels in which the blood-pressure is higher.

II. TECHNIC AND EXPERIMENTS.—The animals were etherized and their abdomens opened by a transverse semicircular laparotomy. The graft of the vena cava to the aorta was composed of four stages.

1. *Extirpation of a Segment of Vena Cava.*—At a short distance below the mouth of the renal veins, the vena cava was dissected and isolated between two ligatures. A venous segment very much longer than the arterial segment to be replaced was resected. It was then washed in Locke's solution, and deposited in a jar of vaseline.

2. *Temporary Hæmostasis, Section, and Resection of the Aorta.*—The abdominal aorta was dissected at the level of the genital arteries. The lumbar collateral branches were forci-pressed or ligated. Between two Crile forceps, a long segment of aorta was isolated and a small part of it resected. As soon as the aorta was cut, the blood was washed out by an injection of Locke's solution. The vessel and the operative region were covered with vaseline, and isolated from the surrounding structures by black Japanese silk towels.

3. *Graft of the Vena Cava.*—The venous segment was removed from its jar, and interposed between the cut ends of the aorta, after the vaseline had been expressed from its lumen. The anastomoses were made by the ordinary method. Straight Kirby needles, No. 16, and fine silk threads, sterilized in vaseline, were used. The ends of the vessels were united by

three retaining stitches and a continuous through-and-through suture. As the diameter of the vena cava was larger than the diameter of the aorta, the calibre of the vena cava was progressively reduced. This could be done easily by leaving a larger distance between the stitches on the vein than on the artery. In order to secure an accurate approximation of the internal surfaces and a narrow scar, the edge of the vein was slightly eversed, the stitches were as loose as possible, and great care was taken not to include any connective tissue in the line of suture.

4. *Re-establishment of the Circulation.*—The Crile forceps placed on the lower end of the aorta was removed, and the blood entered the grafted segment. The sutures and the ligatures of the collateral branches of the vena cava were examined. The anastomoses were slightly compressed with dry gauze pads. The upper forceps were removed and the circulation re-established. After an arteriovenous anastomosis, the lines of sutures leak more or less for a few minutes, after which the hemorrhage stops spontaneously. If after two or three minutes there is still some hemorrhage, one or two stitches are added. The operation must not be ended before it is certain that not a drop of blood is oozing from the lines of suture. When the condition of the circulation was normal, the operation was completed by suture of the lumbar peritoneum and the closing of the abdominal section by four or five planes of suture. The animals were dressed and after a few hours allowed to walk and eat as usual.

Three experiments were performed on two cats and one dog.

Experiment 1: Transplantation of a segment of vena cava between the cut ends of the abdominal aorta.

Large yellow male cat. July 10, 1907. Dissection of the vena cava below the renal arteries. A segment three centimetres long is extirpated. Resection of the right kidney. Dissection and section of the aorta a few centimetres below the renal arteries. Graft between the ends of the segment of vena cava. July 15: Cat in normal condition. Normal pulsations of the femoral arteries. October 1: Cat in excellent health. No change in the femoral pulse. April 15, 1908: Femoral pulse normal. Cat slightly ill. April 25: Cat is sick. Femoral pulse normal. May 1,

1908: Pulse has disappeared. Animal walks normally. May 8: The animal is very sick. No femoral pulse. Killed by chloroform.

Autopsy.—Pyelonephritis, with perinephritic abscess. Obliteration of the transplanted segment by a clot of recent formation which is adherent to the wall. The aorta above and below the transplanted segment is normal. The calibre of the segment is almost the same as it was at the time of the operation. There is no sclerosis of the surrounding connective tissue. The venous wall is about the same thickness as the aortic wall. The internal surface of the lower part of the transplanted segment is smooth and glistening. On the upper part it is covered by a red clot, which is adherent to the wall at about one centimetre below the upper anastomosis. Both anastomoses are in perfect condition, smooth and glistening, and almost invisible. *Histological Examination.*—A small piece of the wall is cut at the level of the lower anastomosis. Section 97. Hæmatoxylin eosin and Weigert's elastic tissue stain. Longitudinal section of the vessel, showing the lower anastomosis and the adjoining parts of the aorta and vena cava. The wall of the vena cava is a little thicker than the wall of the aorta. The aortic wall is normal. Venous wall is composed of connective tissue with a few elongated cells. There are apparently no muscular cells. The union of the vena cava and of the aorta is very smooth. The aortic wall is slightly everted outward at the point of union. Very few elastic fibres in the venous wall, which is almost entirely composed of connective tissue.

Experiment 2: Resection of a small segment of the aorta of a cat. Graft of a segment of vena cava.

Large white male cat, very old. March 24, 1909, 10 A.M. Extirpation of a segment of vena cava of about 35 millimetres, just below the renal vein. Resection of a short segment of aorta at the level of the spermatic arteries. Graft of the vena cava between the cut ends. 3 P.M.: Animal walks about its cage. April 20: Femoral pulse normal. Animal is very fat and in good health. May 20: Animal is sick. Femoral pulse normal. May 25: Animal died.

Autopsy.—Sclerosis of the kidneys. Fatty degeneration of the liver. *Macroscopical Examination.*—The transplanted segment is 34 millimetres long. Its calibre is larger than the calibre of the aorta. It does not seem more dilated than at the time of the operation. The walls of the aorta and vena cava are about of the same thickness. The internal surface is glistening, and slightly irregular. The anastomoses are excellent, without dilatation or stenosis. *Microscopical Examination.*—Hæmatoxylin eosin and Weigert elastic tissue stain. On a cross section of the transplanted segment, at about its middle part, the wall is composed of two parts— external and internal. The external coat is a very thick and well-vascularized adventitia; the internal part is composed of the media and of the intima. The media consists chiefly of very dense connective tissue and of elongated cell nuclei. Longitudinal section of the upper anastomoses shows the media of the aorta slightly bent outward and in perfect union with the vein (Fig. 1). There are no elastic fibres in the venous wall (Fig. 2).

Experiment 3: Resection of a segment of the abdominal aorta patched with peritoneum. Graft of a segment of vena cava.

Middle-sized white bitch. November 24, 1908. Dissection of the abdominal aorta, a piece of which had been resected twenty-two months ago and replaced by a patch of peritoneum. Section of a segment of vena cava below the renal arteries. Resection of the patched segment of the aorta. Graft of the vein between the cut ends of the aorta.

November 25, 1908: Animal in good condition. Her hind legs are swollen. December 5, 1908: Œdema has disappeared almost completely. Animal in excellent condition. July, 1909: Animal normal. Normal femoral pulse. January 13, 1910: Animal died at the farm after an illness of a few days.

Autopsy.—Double pneumonia. Dissection of the aorta and of the venous segment which is 36 millimetres long. Its calibre has not markedly increased. The wall is very much thicker and a little transparent. The internal surface of both vessels is perfectly smooth and glistening, and anastomoses are excellent (Fig. 3). *Microscopical Examination.*—Intima a little thickened. The interstitial connective tissue has very much increased. The muscle-fibres are normal and seem to be increased in number. Very marked thickening of the adventitia (Fig. 4).

III. RESULTS.—The three animals which underwent the transplantation of the vena cava on the abdominal aorta were in normal condition a few hours after the operation and recovered without any complication. In Experiment 3, the posterior limbs of the dog became swollen on account of the ligature of the vena cava but, after a short time, the œdema disappeared completely. No modification of the femoral pulse was observed. The causes, which after several months brought about, directly or indirectly, the death of the animals are completely independent of the operations. In Experiment 1, the cat was chloroformed ten months after the operation because he had developed pyelonephritis. The cat of Experiment 2 was very old. Three months after the operation, he became sick and died. The autopsy showed fatty degeneration of the liver and sclerosis of both kidneys. The dog of Experiment 3 lived in perfect health for fourteen months and then died of pneumonia in a few days.

Therefore, three months, ten months, and fourteen months after the operation the abdominal aorta of the animals and its venous segment were extirpated for the study of the anastomosis and of the venous wall.

Before longitudinal opening of the vessel the anastomoses
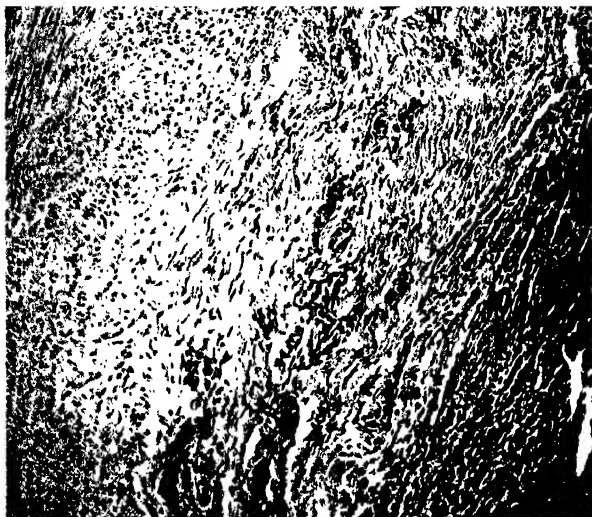
Longitudinal section of the upper arteriovenous anastomosis. Experiment 2

Fig. 2

Same section stained by Weigert method.

FIG. 3.

Segment of vena cava transplanted on the abdominal aorta. Experiment 3. Fourteen months after the operation.

FIG. 4.
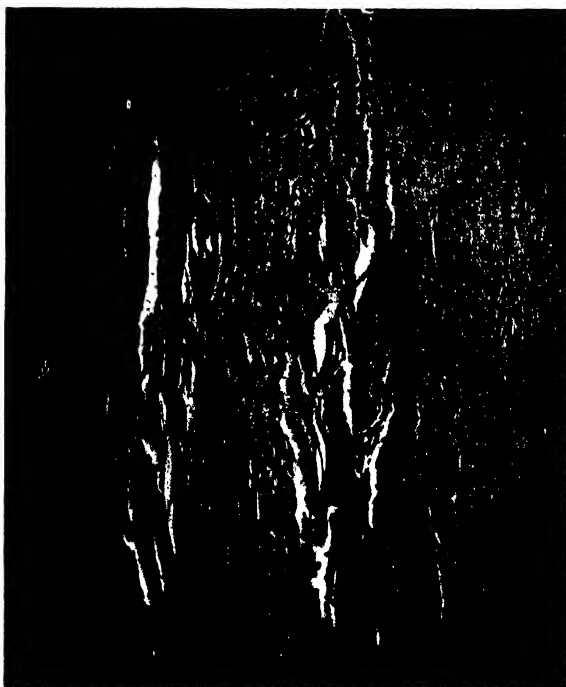
Venous wall, fourteen months after the transplantation. Experiment 3.

could not be precisely located.   There was no hardening of
the wall at their level.   The surrounding connective tissue was
not sclerosed.   In Experiment 1, the segment was occluded
by a clot of recent formation.   But, both anastomoses were
normal.   Their lumen was free.   The scar was indistinct.
The exact location of the anastomosis was determined by the
difference in color between the venous and the aortic walls.
In Experiment 2, there was a linear scar.   In Experiment 3,
the scar was also very narrow and almost invisible (Fig. 3).
But the location of the anastomoses was easily detected because
the aortic wall assumed a white color while the venous wall
was slightly bluish.   The union between the ends of the aorta
and the segment of vena cava was very intimate.   The media
of the aorta bent slightly outward and ended abruptly.   On
its internal surface, the intima increased progressively in thick-
ness, the artery united itself to the vein without interposition
of scar tissue.   The histological appearance of the anastomoses
after the operation shows that the results are definitive (Figs.
1 and 2).   The secondary occlusions observed by Frouin are
certainly the result of a fault of technic.   When an arterio-
venous anastomosis is made by a proper method, no modifi-
cation of its lumen can occur even after a long time.

The venous segment underwent a slight increase of its
calibre and a very marked thickening of its wall (Fig. 3).   In
every experiment its diameter appeared to be slightly enlarged.
The changes of the wall were very marked.   In Experiment 1,
the venous segment was occluded by a clot of recent formation,
which developed after the occurrence of pyelonephritis.   Above
and below the thrombus, the venous wall was smooth and
glistening.   In Experiments 2 and 3, the internal surface
of the wall was perfectly smooth.   There was a slight differ-
ence in color between the aortic and venous walls.   The wall
of the vena cava was a little thicker than the arterial wall.   It
had lost all elasticity, and did not contract when the
circulation was stopped.   It was composed in Experiments
1 and 2 of dense connective tissue, the adventitia and the media
being very much increased.   Development of new elastic fibres
was not observed (Fig. 2).   In Experiment 1, the muscle-

fibre nuclei disappeared. In Experiment 3, the muscle-fibres were found normal (Fig. 4). The thickening of the wall was due chiefly to a very marked sclerosis of the adventitia and of the media. It seemed that the number of the muscle-fibres had increased.

The adaptive changes of the vein begin immediately after its grafting on the artery. The wall thickens. There is, at first, an increase in the connective tissue, and probably also an increase in the number of the muscular fibres. Stich has observed the development of new elastic fibres. I observed it also in a few cases. But often, as it is shown in Experiments 1 and 2, there is no production of new elastic fibres. The elastic framework of the aorta stops abruptly at the point of anastomosis, and the venous wall is seen completely lacking in elastic tissue (Fig. 2). It seems that the first changes undergone by the vein can be compared to hypermyotrophy observed by Russell [14] in the first stage of certain forms of arteriosclerosis. It is a functional hypertrophy due to the increase of blood-pressure. It is not due to a change of nutrition produced by the arterial blood, because I found changes of the same nature in arteries in which the pressure was slightly raised, without any modification of the blood.

It is then certain that the first result of the increase of blood-pressure is an hypertrophy of the wall as it was shown long ago by Adami. [15] The vein has a tendency to become an artery. My experiments demonstrate that it can play perfectly its rôle. But it is possible that in some cases the wall undergoes a progressive sclerosis and that the muscle-fibres disappear completely after a few months. Four months after the transplantation of a segment of jugular on the carotid artery, I extirpated a small part of the wall and sutured the opening. The wall was very thick and composed of a sclerosed media containing normal muscle-fibres and of a greatly hypertrophied adventitia. Twenty months after the operation, the wall was examined again and modified. Sclerosis had increased and all the muscle had disappeared. This change did not interfere at all with the functions of the vena segment. Many experiments have shown that the presence of muscular

or elastic fibres is not necessary to the normal function of an artery. However, sclerosis of the wall may lead to atheromatous changes, although it has not yet been observed. But it will be necessary to keep under observation animals with veins transplanted on arteries for eight or ten years in order to be sure that these degenerative changes do not occur. The knowledge of the remote results of these operations will decide whether fresh veins or arteries, fresh or preserved in cold storage, must be selected as grafts.

IV. CONCLUSIONS.—The transplantation of the vena cava on the aorta is not a dangerous operation. The three animals operated on recovered without incident.

The venous wall reacts against the arterial blood-pressure by thickening its wall.

The condition of the venous wall and of the anastomoses examined fourteen months after the operation shows that, for a long time, a segment of vena cava can functionate as a part of the abdominal aorta.

It is probable that in the treatment of aneurism, rupture of large arteries, embolus, and localized arteritis, the transplantation of venous segments can be used safely, but it must be emphasized that without a proper technic, the results of the operation will be disastrous.

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THE PARTIAL HYDROLYSIS OF PROTEINS.

II. ON FIBRIN-HETEROALBUMOSE.

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(Received for publication, August 15, 1910.)

The study of partial cleavage has proved of great service for the interpretation of the constitution of many complex substances. The value of the same method for the elucidation of the structure of the protein molecule has been discussed by one of us some time ago.[1] Here, again, we wish to emphasize that we are fully aware of the difficulties which one encounters when attempting to separate the individual products of partial hydrolysis of proteins, both of colloidal and crystalline nature. However, we feel confident that the classical methods of the schools of Kühne, of Chittenden, of Hofmeister, and the recently added method of Siegfried and his co-workers—though from a chemical standpoint imperfect—still remain of great service for the study of the chemical structure of the protein molecule. The different albumoses may not represent chemical individuals; but even if they are mixtures, they are mixtures similar within each fraction not only by the physical properties, but also by the chemical composition of the parts of the mixture. This can be stated with certainty in regard to the so-called primary digestion products, the hetero- and the proto-albumoses. The work of Adler[2] and of Birchard[3] in Siegfried's laboratory has contributed much evidence in support of this view. The work undertaken in this laboratory several years ago, and which was planned to be a systematic study of every individual fibrin-albumose, and of the products of partial hydrolysis of the

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[1] P. A. Levene, this *Journal*, i.

[2] Dissertation, Leipzig, 1907.

[3] Dissertation, Leipzig, 1909.

same, was for a time interrupted. This interruption was caused principally by work on the improvement in the methods of analysis of the products of protein hydrolysis. Considerable advance in the methods of analysis have been made through the efforts of Osborne and his co-workers, of Siegfried and his pupils, and through the work done in this laboratory.

It was therefore concluded to resume the work on the products of partial hydrolysis of fibrin. The aim of the work is to find evidence which will lead to a definite solution of the following queries.

First, whether or not on partial hydrolysis the protein molecule is decomposed into large fragments—which appear in the form of albumoses.

Second, whether or not the partial hydrolysis of proteins proceeds by degrees, in a manner such that only individual amino-acids, or simple peptides are detached from the original molecule, so that by degrees the number of amino-acids contained in it decreases, and the protein is transformed through the more complex albumoses into the simpler, and finally into peptones, peptides, and amino-acids.

Third, whether or not the two processes occur simultaneously. In that case the task will arise to trace the relationship of the simpler albumoses or peptones to one or the other of the more complex substances.

The present communication is limited to the work on the preparation and on the hydrolysis of the hetero-albumose.

PREPARATION OF THE HETERO-ALBUMOSE.

The method of preparation consisted in a combination of each of the methods as worked out by Kühne and Pick, and was the same as that adopted by one of us[1] in a former investigation.

The details of the method are as follows:

A 10 per cent solution of Witte's peptone was carefully neutralized with dilute sulphuric acid, allowed to stand over night, and the undissolved residue filtered off. It was later found that the solution could be centrifugalized

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[1] P. A. Levene: this *Journal*, i, p. 1.

with advantage and consequent saving of considerable time. To the clear solution obtained by either method was added an equal volume of a concentrated solution of ammonium sulphate. The primary albumoses were filtered off, washed with half-saturated ammonium sulphate solution, and twice reprecipitated in half the former dilution. The hetero- was separated from the proto-albumose by adding an equal volume of 95 per cent alcohol, allowing to stand two days, and filtering off the hetero-albumose by the aid of suction. The product so obtained was carefully washed with 50 per cent alcohol, dissolved in a volume of warm water equal to half of that formerly employed, and twice reprecipitated by the addition of an equal volume of 95 per cent alcohol. The final product was dissolved in warm water, a little ammonium sulphate being added to aid the solution. The product was then subjected to dialysis until no more sulphate could be detected by barium chloride. By this process a fine granular substance was obtained which was finally washed by decantation in a large excess of distilled water. This method involves a very large loss of material, but it was only in this way that a pure product could be obtained.

Properties.

The albumose obtained in this way was extremely insoluble in water; when suspended in 4 liters of water and after intermittent stirring for 12 hours, 100 cc. of the supernatant liquid contained only 0.0042 gm. of nitrogen or 0.025 gm. of albumose. For analysis a sample was dried by heating to constant weight under diminished pressure at the temperature of boiling water. The following results were obtained:

0.2056 gm. substance gave 0.3734 gm. CO_2 and 0.1220 gm. H_2O.
0.1628 gm. of the substance employed for a nitrogen estimation after Kjeldahl, required 19.15 cc. $\frac{N}{10}$ H_2SO_4.

These results agree fairly well with those obtained by Adler[1] with Pick's hetero-albumose, as can be seen from the following table:

Adler's Analysis.		*Present Analysis.*	
	Per Cent		Per Cent
C =	48.18	C =	49.52
H =	6.63	H =	6.64
N =	16.00	N =	16.46

For further identification the rotatory power of the substance dissolved in 5 per cent ammonium sulphate solution was deter-

[1] Dissertation, Leipzig, 1907.

mined. For this determination very careful drying is necessary, as prolonged heating at temperatures over 100° C. tends to change the substance into a form insoluble even in salt solution. The albumose was first dried two days under diminished pressure over sulphuric acid, then for two days under diminished pressure at the temperature of boiling chloroform, and finally to constant weight at the temperature of boiling alcohol.

0.1615 gm. substance, dissolved in 25 cc. rotated − 0.84° in 1.855 gm. tube at t = 20° C. Thus:

$$[\alpha]_D^{20} = - 70.11°.$$

This is in close agreement with the value found by Adler, − 70.69° for an ammoniacal solution of the hetero-albumose.

Primary Amino Nitrogen: 0.2393 gm. albumose treated with nitrous acid gave 4.40 cc. of nitogen gas at 21°, 756 mm.

Amino N = 1.03 per cent = 6.3 per cent of the total N.

HYDROLYSIS.

Glutaminic Acid and Esterified Acids.

One-hundred and twenty-nine grams of the hetero-albumose were hydrolyzed by boiling 15 hours, with 20 per cent hydrochloric acid. The solution was concentrated, saturated with hydrochloric acid, and left for 10 days in the refrigerator. The glutaminic hydrochloride was filtered on asbestos and recrystallized. 10.83 gms. of the pure hydrochloride, equivalent to 8.67 gms. of glutaminic acid, were obtained.

Analysis: 0.4370 gm. subst.; 23.80 cc. $\frac{N}{10}$ Ag NO$_3$.

	Calculated for $C_5H_9O_4N.HCl$:	Found:
Cl...........................	19.31 per cent	19.32 per cent

The mother liquors were esterified according to Fischer's method, three crops of esters being freed with barium hydrate and extracted by the method of Levene and Van Slyke.[1] The esters were distilled, using H_2SO_4 in place of liquid air refrigeration, to absorb uncondensed vapors.[2] The esters boiling above 90° were not

[1] Levene and Van Slyke: this *Journal*, vi, p. 391. 1909.
[2] Levene and Van Slyke: *Biochem. Zeitschr.*, x, p. 214, 1908.

distilled, but worked up as recently proposed by Osborne and Jones.[1] The results were satisfactory.

The esters were divided into the following fractions by distillation.

	TEMPERATURE OF VAPORS.	PRESSURE.	WEIGHT OF ESTERS.
	degrees	*mm.*	*gm.*
I.................	to 60	12.0	27.8
II.................	to 90	0.4	32.0
III...............	Undistilled	....	32.3
Total			92.1

Fraction I was chiefly alcohol. It yielded by crystallization 0.95 gm. of a mixture containing 14.18 per cent N, and on evaporating the mother liquors to dryness, 0.96 gm. of more soluble acids. The first crop was worked up with the alanin-valin mixtures from Fraction II. The second crop was combined with the more soluble portion of Fraction II, and extracted with absolute alcohol to remove prolin.

Leucin-Valin Sub-Fraction.

Fraction II yielded three fractions by crystallization, which nitrogen determinations by the nitrous acid method[2] showed to be mixtures of valin and leucin, the weights and nitrogen contents being 2.96 gms., 11.07 per cent N; 4.00 gms., 11.34 per cent N; 4.12 gms., 11.71 per cent N. These fractions were combined, and the leucin, isoleucin and valin determined by precipitating the leucin isomers as lead salts, and determining the proportion of the two isomers by the rotation of their mixture in 20 per cent HCl.[3] One-half of the mixture was used for the lead separation. It yielded 6.91 gms. of the lead salt of leucin, equivalent to 7.76 gms. of the leucin isomers from the entire mixture.

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[1] *Amer. Journ. of Physiol.*, xxvi, p. 212, 1910.

[2] D. D. Van Slyke: Method for Determination of Amino Nitrogen. *Proceedings Soc. Exp. Biol. and Med.*, December 15, 1909; *Ber. d. d. chem. Ges.*, xliii.

[3] Levene and Van Slyke: Analysis of the Leucin Fraction of Proteins, this *Journal*, vi, p. 391, 1909.

Analysis: 0.2958 gm. subst.; 0.1924 gm. $PbSO_4$:
0.2158 gm. subst.; 22.5 cc. N at 18°, 756 mm. (nitrous acid method).

	Calculated for $Pb(C_6H_{12}O_2N)_2$:	Found:
Pb........................	.44.29 per cent	44.32 per cent
N.........................	6.00 per cent	5.92 per cent

The leucin and isoleucin were freed from their salts as described by Levene and Van Slyke, and the rotation of the mixture taken in 20 per cent HCl.

0.2632 gm. subst; 5.016 gm. solution; concentration, 5.15 per cent; rotation in 1 dm. tube, + 1.52.°

$$[\alpha]_D^{20} = + 26.32°.$$

Calculated from the rotation, the mixture contained 50.8 per cent of *l*-leucin, 49.2 *d*-isoleucin, or 3.94 and 3.82 gms. respectively. The mixture of the free leucin isomers gave the following figures on analysis:

0.1570 gm. subst.; 30.34 cc. N at 24°, 760 mm. (nitrous acid method).

	Calculated for $C_6H_{11}O_2N$:	Found:
N.........................	10.69 per cent	10.78 per cent

The filtrate from the leucin lead salts yielded 1.46 gms. of valin, equivalent to 2.92 gms. for the entire portion.

Analysis: 0.1141 gm. subst.; 25.30 cc. N at 30°, 756 mm. (nitrous acid method).
0.1594 gm. subst.; 0.3082 gm. CO_2; 0.1490 gm. H_2O.

	Calculated for $C_6H_{11}NO_2$:	Found:
N.........................	11.96 per cent	11.88 per cent
C.........................	51.24 per cent	51.33 per cent
H.........................	9.47 per cent	9.64 per cent

Valin-Alanin Sub-Fraction.

The mother liquors from the above crystillization fractions were freed from prolin by extraction in the usual manner with absolute alcohol, then submitted to fractional crystalization.   1.65 gms.

more of valin was obtained, making the entire yield of pure valin 4.57 gms.

Analysis: 0.1340 gm. subst.; 30.5 cc. N at 32°, 756 mm. (nitrous acid method).

	Calculated for $C_5H_{11}O_2N$:	Found:
N........................	11.96 per cent	12.08 per cent.

Rotation in 20 per cent HCl: 0.2460 gm. substance; 5.114 gm. solution; concentration, 4.81 per cent; rotation in 1 dm. tube, $+ 1.25.°$

$$[\alpha]_D^{20} = + 23.63°.$$

3.61 gms. of recrystallized alanin were obtained from the valin mother liquors.

Analysis: 0.1161 gm. subst.; 34.7 cc. N at 32°, 756 mm.
0.1388 gm. subst.; 0.2054 gm. CO_2; 0.0955 gm. H_2O.

	Calculated for $C_3H_7O_2N$:	Found:
N........................	15.73 per cent	15.85 per cent
C........................	40.42 per cent	40.36 per cent
H........................	7.93 per cent	7.69 per cent

1.10 gms. of a mixture of alanin and valin (C = 44.81, H = 8.58) were obtained which could not be separated. No glycocoll could be obtained by the carbamino method of Siegfried[1] although this method was tried out on mixtures of glycocoll and *d*-alanin and found to be fully as satisfactory as Siegfried claims. For example from a mixture of 0.37 gm. glycocoll and 1.00 gm. *d*-alanin in 50 cc. baryta water, 0.28 gm. of pure glycocoll was obtained (N = 18.90, calc. 18.67). The failure to detect glycocoll by this method therefore indicates that very little if any was present. No fractions which from analysis appeared to be mixtures containing glycocoll could be obtained on recrystallization.

Prolin.

The amino-acids completely soluble in cold alcohol were dissolved to 100 cc. in water, and the prolin determined by the method of Van Slyke.[2]   Kjeldahls on 5 cc. samples required 35.75 − 35.65

[1] *Ber. d. d. chem. Ges.*, xxxiv, p. 400.
[2] *Ber. d. d. chem. Ges.*, xliii.

cc. of $\frac{N}{10}$ H_2SO_4 for titration, indicating 1.000 gm. of nitrogen in the entire solution. Determination of the primary amino nitrogen in 10 cc. of the solution gave 71.10 cc. of nitrogen gas at 21°, 762 mm., indicating that 0.4025 gm. of the nitrogen came from the ordinary aliphatic amino-acids, 0.5975 gm. from the prolin in the mixture. This corresponds to 4.92 gms. of prolin.

The 80 cc. of the solution, remaining after removal of the samples for the above determinations, was racemicized, boiled with CuO, and the prolin obtained as crystalline d-l copper salt. 3.77 gms. were regained, equivalent to 60.6 per cent of the amount of prolin calculated.

Analysis: 0.3784 gm. subst.; loss at 100°, 0.0385 gm.; 11.70 cc. $\frac{N}{10}$ ammonium sulphocyanide (Volhard titration), 0.2310 gm. subst.; amino nitrogen, 4.70 cc. at 19°, 760 mm.

	Calculated for $Cu(C_5H_8O_2N)_2.2H_2O$:	Found:
H_2O..........................	10.99 per cent	10.72 per cent
Cu...........................	19.41 per cent	19.72 per cent
Amino N.....................	0.00 per cent	1.16 per cent

After recrystallizing three times the prolin salt was obtained almost free from amino nitrogen. The amino nitrogen test is by far the most delicate for the purity of .prolin obtained from proteins.

Analysis: 0.3484 gm. subst.; 0.0375 loss at 100°; 10.70 cc. $\frac{N}{10}$ ammonium sulphocyanide. 0.1906 gm. subst.; 0.60 cc. N at 30°, 760 mm. (nitrous acid method).

	Calculated for $Cu(C_5H_8O_2N)_2.2H_2O$:	Found:
H_2O..........................	10.99 per cent	10.76 per cent
Cu...........................	19.40 per cent	19.51 per cent
Amino N.....................	0.00 per cent	0.17 per cent

The mother liquors from the first crop of copper salt were freed from copper by hydrogen sulphide, and the amino-acids crystallized from dilute alcohol. 1.35 gms. were thus obtained, a mixture of alanin and valin (N = 13.72 per cent). The mother liquors were reconverted into copper salts, and yielded 0.57 gm. more of prolin salt (H_2O = 11.04, Cu = 19.62, amino N = 0.72), making the total crystallized copper salt regained 69.7 per cent of that calculated by the nitrogen determinations.

Phenylalanin.

Fraction III of the esters was dissolved in water and the phenylalanin ester extracted with ether in the usual manner.   The ethereal extract contained practically all of the coloring matter.   It was decolorized with charcoal, and the phenylalanin obtained as hydrochloride from aqueous solution after saturation with HCl.   3.85 gms. of the hydrochloride, equivalent to 3.16 gms. of phenylalanin, were obtained.

Analysis: 0.3201 gm. subst.; 16.05 cc. $\frac{N}{10}$ silver nitrate.

	Calculated for $C_9H_{11}O_2N$:	Found:
Cl	17.58 per cent	17.76 per cent

Glutaminic and Aspartic Acids from Esters.

The esters not extracted from water solution by ether were hydrolyzed with barium hydrate, as usual, the solution was freed from barium with sulphuric acid, and the glutaminic acid crystallized as hydrochloride.   4.47 gms. equivalent to 3.59 gms. of glutaminic acid were obtained.

Analysis: 0.2970 gm. subst.; 16.30 cc. $\frac{N}{10}$ silver nitrate.
0.1430 gm. subst.; 19.80 cc. N at 25°, 760 mm. (nitrous acid method).

	Calculated for $C_5H_9O_4N.HCl$:	Found:
Cl	19.31 per cent	19.45 per cent
N	7.63 per cent	7.68 per cent

The mother liquors were freed from HCl by concentration *in vacuo*, followed by use of silver sulphate, hydrogen sulphide, and an equivalent of barium hydrate to remove SO_4.   The solution was concentrated and mixed with several volumes of alcohol.   6.10 gms. of aspartic acid crystallized on standing in the refrigerator.

Analysis: 0.1297 gm. subst.; 22.30 cc. N at 29°, 758 mm. (nitrous acid method).
0.1272 gm. subst.; 0.1643 gm. CO_2; 0.0614 gm. H_2O.

	Calculated for $C_4H_7O_4N$:	Found:
N	10.54 per cent	10.58 per cent
C	36.06 per cent	36.28 per cent
H	5.30 per cent	5.40 per cent

No serin could be obtained from the mother liquors.

Prolin, Alanin, and Glycocoll from Unextracted Ester Residues.

The barium residues left after the third extraction of esters were freed from hexone bases by phosphotungstic acid, and from sulphate, chloride, and excess phosphotungstate by barium hydrate and silver sulphate. The solution of amino-acids was concentrated, and 1 gm. of tyrosin filtered off. The other amino-acids were all converted into copper salts; the salts proved extremely soluble in water. Their solution was brought to 150 cc. and 600 cc. of alcohol added. The precipitated salts dried at 100° *in vacuo* weighed 15.6 gms.; the salts soluble in 80 per cent alcohol, 8.5 gms.

The insoluble fraction was changed back to free acids. They refused to be crystallized from water or dilute alcohol. An attempt was made to obtain the picrate of glycocoll by Levene's method.[1] However, in place of the usual glycocoll picrate 1.56 gms. of a picrate differing in composition and in properties from the glycocoll compound were obtained. It was attempted to purify the substance by recrystallization from an alcoholic solution of picric acid. A great part of the substance remained in solution, as only 0.4 gm. was obtained on recrystallization. This picrate was not explosive and contained only traces of mineral impurities. Tested in the usual manner for pyrrol, it gave a very definite positive reaction, melted between 235–240° C. (corr.) with decomposition and evolution of gas.

Analysis: 0.1248 gm. subst.; 15.7 cc. N(over 50 per cent KOH) at 29°, 763 mm.

	Calculated for $C_2H_5NO_2.C_6H_2(NO_2)_3OH$:	Found:
N	18.42 per cent	14.28 per cent

Thus the substance was not glycocoll picrate. Lack of material did not permit of a detailed study of the substance at the present moment.

In the mother liquors of the first picrate on standing a second precipitate formed, about 0.80 gm. in weight with the properties of the glycocoll compound. M.p. = 190° C. (corr.) sharp.

[1] P. A. Levene, this *Journal*, i, p. 463.

Analysis: 0.120 gm. subst.; 20.0 cc. N (over 50 per cent KOH)
at 30°, 757 mm.).

	Calculated for $C_3H_5NO_2.C_6H_2(NO_2)_3OH$:	Found:
N	18.42 per cent	18.80 per cent

The copper salts soluble in 80 per cent alcohol were extracted with absolute alcohol. The soluble salts were reconverted into amino-acids, racemicized, changed back to Cu salts, and recrystallized from water. 0.75 gm. of anhydrous prolin copper salt, equivalent to 0.59 gm. prolin, was obtained. The product was not entirely pure, but was of the characteristic violet color when dried at 100°, and gave the following analysis.

0.3490 gm. subst.; 11.10 cc. $\frac{N}{10}$ ammonium sulphocyanide.
0.2200 gm. subst.; 9.30 cc. N at 32°, 756 mm. (nitrous acid method).

	Calculated for $Cu(C_5H_8O_2N)_2$:	Found:
Cu	19.40 per cent	20.21 per cent
Amino N	0.00 per cent	2.15 per cent

The copper salts soluble in 80 per cent alcohol, but insoluble in absolute, were decomposed with H_2S, and the amino-acids crystallized from dilute alcohol in the hope of obtaining serin. Instead of serin, however, 0.75 gm. of alanin was obtained.

Analysis: 0.1339 gm. subst.; 0.0958 gm. H_2O.

	Calculated for $C_3H_7O_2N$:	Found:
C	40.42 per cent	40.23 per cent
H	7.93 per cent	8.00 per cent

Tyrosin.

Thirty-one grams of hetero-albumose were hydrolyzed with 20 per cent hydrochloric acid. The acid was removed by concentration *in vacuo* and the use of silver sulphate. The solution of amino-acids was concentrated *in vacuo* until crystallization began. 1.025 gms. cf tyrosin were obtained.

Analysis: 0.1586 gm. subst.; 0.3475 gm. CO_2; 0.0881 gm. H_2O.
0.1235 gm. subst.; 16.35 cc. N at 21°, 768 mm. (nitrous acid method).

	Calculated for $C_9H_{11}O_2N$:	Found:
C	59.67 per cent	59.73 per cent
H	6.08 per cent	6.21 per cent
N	7.73 per cent	7.58 per cent

By further concentration a second crop cf 0.053 gm. was obtained, making the total yield 1.078 gms., or 3.48 per cent.

Analysis: 0.0530 gm. substance; 0.1151 gm. CO_2; 0.0300 gm. H_2O.
C, 59.23 per cent; H, 6.29 per cent.

Hexone Bases by the Osborne Modification of Kossel's Method.

18.80 gms. of albumose were hydrolyzed; and the hexone bases precipitated by phosphotungstic acid at 2 liters dilution in the presence cf 5 per cent sulphuric acid. The precipitate was freed from phosphotungstate and sulphate by means of barium hydrate, and the histidin, arginin, and lysin determined by the Kossel-Patton method as modified by Osborne, Leavenworth and Brautlecht.[1]

The histidin solution was brought to 25 cc.; determinations were made of total nitrogen by the Kjeldahl method, and of primary amino nitrogen by the nitrous acid method. As has been shown,[2] in pure histidin the ratio, total nitrogen: amino nitrogen, is 3:1, and consequently small amounts of histidin in pure solution can be analyzed by determining this ratio. The following results were obtained on the histidin solution:

Amino N: 10 cc. solution; 11.85 cc. N at 29°, 758 mm. Amino N in total 25 cc. is 0.01605 gm.
Total N: 15 cc. solution; 20.35 cc. $\frac{N}{10}$ H_2SO_4. Total N in 25 cc. solution is 0.0476 gm.
Total N: Amino N = 2.97:1

The histidin solution was evidently pure. The total nitrogen corresponds to 0.176 gm. histidin, or 0.93 per cent.

The arginin solution was brought to 250 cc. volume. The ratio, total N: amino N, is 4:1 in the case of arginin. Determina-

[1] *Amer. Journ. of Physiol.*, xxiii, p. 180, 1908.
[2] Van Slyke: *Proc. Soc. Exp. Biol. and Med.*, Dec. 15, 1909.

tion of amino and Kjeldahl nitrogens serves here to check the purity of the arginin solution. Also, it has been found that one-half of the arginin nitrogen is quantitatively evolved in the form of ammonia during 6 hours boiling with 25 per cent NaOH under a reflux. The greater part of the ammonia diffuses into standard H_2SO_4 in a Folin 3-bulb tube at the top of the condenser, the remainder being distilled off later after addition of water to the alkaline solution. The following determinations were made on the arginin solution:

Total N: 20 cc. solution; 23.50 cc. $\frac{N}{10}$ H_2SO_4. Total N = 0.4010 gm.
Amino N: 10 cc. solution; 9.70 cc. N at 25°, 764 mm. Amino N = 0.1353 gm.
Arginin N[1]; 40 cc. solution; 19.05 cc. $\frac{N}{10}$ H_2SO_4. Arginin N = 0.3330 gm.
N H_3 ; 40 cc. solution; boiled with Mg O; 0.00 cc. $\frac{N}{10}$ H_2SO_4. NH_3 absent.

The ratio, total N: amino N, is lower than 4:1, and the arginin determination indicates that only 83.1 per cent of the total nitrogen present was in the form of arginin. Calculated on this basis, the arginin present was 1.036 gms., or 5.50 per cent.

The solution not used in the above determinations was concentrated and treated with an equivalent of picrolonic acid in alcoholic solution. 0.9 gm. of arginin picrolonate were obtained.

Analysis: 0.1440 gm. subst.; 0.2297 gm. CO_2; 0.0696 gm. H_2O.

	Calculated for $C_{16}H_{22}N_8O_7$:	Found:
C	43.82 per cent	5.05 per cent
H	43.50 per cent	5.41 per cent

From the lysin solution 2.44 gm. of lysin picrate were obtained, equivalent to 0.95 gm. of lysin, or 5.06 per cent. The picrate was recrystallized from water, and gave the following figures on analysis.

0.1517 gm. subst.; 20.4 cc. N at 18°, 746 mm. (nitrous acid method).

	Calculated for $C_{12}H_7N_5O_9$:	Found:
Amino N	7.47 per cent	7.58 per cent

[1] Van Slyke: *Proc. Soc. Exp. Biol. and Med.*, May 18, 1910. The decomposition of nearly one-half the arginin nitrogen into ammonia was noted by Osborne, Leavenworth and Brautlecht. The above method is quantitative.

Determination of the Hexone Bases and Nitrogen Distribution by the Method of Van Slyke.[1]

2.07 gms. of albumose containing 0.3413 gm. of nitrogen, were hydrolyzed with 20 per cent hydrochloric acid, the acid removed as completely as possible by evaporation, and the *ammonia* distilled *in vacuo* with an excess of barium hydrate solution. It neutralized 20.10 cc. of $\frac{N}{10}$ H_2SO_4.

The solution was acidified with the H_2SO_4, the *melanin* removed by adsorption with Ag Cl, and determined by Kjeldahl. 19.75 cc.$\frac{N}{10}$ H_2SO_4 were neutralized.

The filtrate was brought to 100 cc. Portions of 5 çc. each were taken for determination of total and amino nitrogen.

Total Nitrogen: 10.10, 10.25 cc.$\frac{N}{10}H_2SO_4$; average 10.18. Total N = 0.2856 gm.
Amino Nitrogen: 18.55, 18.70 cc. N at 21.5°, 764 mm. Amino N = 0.2116 gm.

To the remaining 80 cc. 4 cc. of concentrated sulphuric acid and 60 cc. of a 20 per cent solution of phosphotungstic acid in 5 per cent H_2SO_4 were added. The mixture was made up to 200 cc. volume and heated until the precipitate was nearly dissolved, then allowed to stand three days. The phosphotungstates of arginin, histidin, lysin and cystin are precipitated under these conditions. The precipitate was washed with a solution containing 5 per cent H_2SO_4 and 2 per cent phosphotungstic acid and decomposed with a slight excess of baryta water. The solution of bases was freed from Ba with CO_2 and brought to a volume of 50 cc. 10 cc. were used for Kjeldahl and amino determinations, in each case the remaining 30 cc. being used, first to determine the arginin by alkaline decomposition as already described (p. 281), and then to determine the cystin sulphur by fusion with KNO_3. The results were:

Phosphotungstate Precipitate.

Total N; 10 cc. solution: 8.25 cc. $\frac{N}{10}$ H_2SO_4. Total N = 0.0722 gm. = 21.15 per cent. of the total N.

Amino N: 10 cc. solution, 11.40 cc. N at 24°, 754 mm. Amino N = 0.0394 gm.

Arginin: 30 cc. solution; 6.80 cc. $\frac{N}{10}$ H_2SO_4. Arginin N = 0.0397 gm. = 11.62 per cent. of the total N.

The non-amino nitrogen ($\frac{3}{4}$ of arginin N $+ \frac{2}{3}$ of histidin N) is 0.0722 − 0.0394 = 0.0328 gm.   Subtracting three-fourths of the arginin N from the non-amino N, gives 0.0031 gm. as the non-amino histidin N, or 0.0046 gm. as the total histidin N, 1.34 per cent of the total nitrogen.

The difference (lysin and cystin N) between the total "basic" N and the histidin N + arginin N is 0.0279 gm. The 30 cc. of solution used for arginin and cystin determinations gave, for the latter 0.0790 gm. $BaSO_4$, indicating 0.0099 gm., 2.90 per cent, of cystin N.   This leaves 0.0180 gm., or 5.28 per cent, for the lysin N.

By subtracting the nitrogen in the phosphotungstic precipitate from that found in the solution before precipitation, the nitrogen of the "mono-amino acid" fraction is obtained.   This method avoids the difficulties of Kjeldahling solutions containing phosphotungstic acid. The total N of this fraction is 0.2134 gm. = 62.54 per cent.   Of this, 0.1722 gm., or 50.48 per cent, is amino nitrogen, 0.0412 gm., or 12.06 per cent, is non-amino nitrogen, consisting of the nitrogen of prolin, oxyprolin, one-half the tryptophan and perhaps some yet unknown acid or acids.

The results are summarized as follows:

	GRAMS NITROGEN	PER CENT OF TOTAL NITROGEN	
Ammonia	0.0281	8.23	
Melanin	0.0276	8.08	
Arginin	0.0397	11.62	
Histidin	0.0046	1.35	21.15
Lysin	0.0180	5.28	
Cystin	0.0099	2.90	
Amino N in phosphotungstic filtrate	0.1722	50.48	62.54
Non-amino N in phosphotungstic filtrate	0.0412	12.06	

The hexone base determinations, calculated for percentages of amino-acids in the dry albumose, compare as follows with those obtained by the older method.

	KOSSEL-PATTON-OSBORNE METHOD	NEW METHOD	MEAN
Arginin	5.50	5.96	5.73
Histidin	0.95	0.82	0.89
Lysin	5.06*	4.54	4.80

* Lysin was calculated from unrecrystallized picrate, so the result may be slightly high.

Correction for the solubility of the phosphotungstates, which is practically the same in both methods, as the concentrations at precipitation were alike, increases the average arginin to 6.35 per cent, the histidin to 1.76 per cent.

The results of the hydrolysis are summarized in the following table, expressed in grams of amino-acid from 100 gms. of albumose.

Glutaminic acid	9.51	Aspartic acid	4.73
Leucin	3.05	Glycocoll	0.15
Isoleucin	2.96	Tyrosin	3.48
Valin	3.54	Arginin	6.35
Alanin	3.39	Histidin	1.76
Valin-Alanin Mixture	1.86	Lysin	4.80
Prolin	4.27	Cystin	4.10
Phenylalanin	2.45	Ammonia	1.65
		Total	58.05

CULTIVATION OF ADULT TISSUES AND ORGANS OUTSIDE OF THE BODY *

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The solution of many problems of human pathology depends, in a large measure, on the finding of the still unknown physiologic laws of generation, growth and evolution of cells. We must, therefore, develop new methods which permit the discovery of these laws. A few weeks ago, we began to investigate systematically one of these future methods, namely, the cultivation of adult tissues outside of the body. The starting point of our researches was the beautiful work of Harrison on the embryonic tissues of the frog. Some years ago, Harrison observed the development of nerves from the central nervous system of frog embryos cultivated in a drop of lymph. In 1910, Burrows studying with Harrison improved very much this method and adapted it to embryonal tissues of warm-blooded animals. He succeeded in cultivating nerves and mesenchymatous cells of sixty-hour chick embryos.

Then, at the Rockefeller Institute, we tried to develop on this basis a general method which would be applicable to the adult tissues of the mammalia and thus to determine some of the laws of cellular physiology.

The experiments on which we wish to report now were performed on dogs and cats and an adult frog and consisted of extirpating small fragments of tissues of an animal, inoculating it aseptically into a plasmatic medium taken from the same animal, and sealing the materials in hollow glass slides. The slides were placed in an incubator, maintained at a constant temperature

of 37 C. The microscope was also placed in a special thermostat which was kept at this temperature. The growth of the cells could, therefore, be observed, over a period of time, with the microscope, kept itself at the body temperature, and the multiplication of cells directly seen.

The plasmatic media were inoculated with many tissues or organs, of which all were found to multiply or grow. The cultures of the different tissues—as we shall call them—contain common characteristics. The time of the beginning of cellular proliferation depends on the nature of the tissue, the age of the animal and other more or less important factors. In the cultivation of glandular organs of adult dogs, the vegetation starts after thirty-six or forty-eight hours. But, if the young animal is only a few days old, new cells appear in the culture after ten or twelve hours. Four or five days after the inoculation of the medium, the cultures of thyroid, kidney, suprarenal, etc., are in full activity, and remain in this condition as long as the medium allows it. Tissues like cartilage or peritoneum grow, at first, very slowly. After three days, there are in the cultures very few new cells. But about one week after the inoculation, the cultures become very much more active, and are in full vegetation after about nine or ten days. There are also some analogies between the morphologic characters of the cultures of various tissues and organs. For all tissues, the first indication of growth is the appearance on the edges or the surface of the specimen of a few small and regular granulations. These granulations consist of the cytoplasm of cells, the nucleus and nucleoli of which soon become visible. The cells belong to two general types, spindle and polygonal. The spindle cells appear ordinarily at first and their morphology is about the same in all tissues, bone marrow or kidney, thyroid or cartilage. They are long and slender and radiate from the fragment of tissue or organ through the plasmatic medium. They are derived probably from the connective tissue framework of the organ. At the same time, or a little later, the cells of the second type appear. They are polygonal or multipolar cells in form, but their morphology varies widely according to each tissue and organ. They seem in part to be differ-

entiated cells of epithelial nature. Cartilage produces cartilaginous cells, and thyroid generates cells which look like thyroid cells. Even in the renal cultures, this second type of cells congregates in tubular formations. By using a suitable technic, we can control the growth of one or another of these types. A small fragment of thyroid cleanly cut produces mainly spindle cells, while in tissues more finely divided (scrapings), epithelial-like cells appear.

In the first part of the work we found and studied the growth of adult tissues outside of the body. In the second part we attempted to cultivate thyroid cells in series, and also to activate the growth of a tissue by passage from one plasmatic medium to another. Connective tissue, cartilage, peritoneum, bone marrow and bone, skin, cornea, mucous membrane of the tongue, thyroid gland, spleen, suprarenal gland, kidney, pancreas, testicle and ovary were all cultivated successfully.

CULTIVATION OF TISSUES

Arterial Sheath.—Three days after inoculation of a fragment of arterial sheath, very delicate palm-like cells appeared on the edge of the tissue and ramified through the plasmatic medium in long filaments ending in spindle cells. Vegetation was very weak and stopped entirely after a few days.

Connective Tissue.—Most of the cultures of connective tissue remained inactive.

Conjugal Cartilage.—This started also to grow on the third day. For about one week, very few spindle and spider like cells were found slowly wandering along the edges of the cartilage. From the upper pole of the fragment of tissue, a mass of cartilage protruded and invaded the plasmatic medium. After a few days, it became so large that it could be seen by the naked eye. Progressively, the increase in size became faster. Many irregular cells with long arms now appeared in the plasma about the old cartilage.

After fourteen days, the culture was in full activity, and the old cartilage had thus increased outside of the body two millimeters in length.

Peritoneal Endothelium.—This underwent also a slow evolution. For several days, there were only a few beautiful and irregularly-shaped cells along the edges of the

tissue. After a week they began to multiply more actively and many very large cells resembling endothelial cells slowly moving through the clear plasmatic medium were directly observed under the microscope. On the sixteenth day, the culture was still in full vegetation.

Bone Marrow.—During the first hours of the cultivation of fragments of bone marrow and bone, the anatomical elements began to wander away from the tissue. After three or four days, the little pieces of bone hidden in the bone marrow became visible, because almost all the cells had invaded the plasmatic medium. Around the tissue, there were radiating spindle cells and many red blood corpuscles. Leukocytes with active ameboid motion and large cells with granular cytoplasm and long pseudopodia had reached the remotest part of the medium. A few large spindle cells were seen crawling along the edges of the fragments of bone.

Epidermis.—We studied the growth of epidermis by cultivating fragments of the skin of an adult frog. Masses of epithelial cells appeared on the edges of the cutaneous fragments after twelve or twenty-four hours. They grew very rapidly. After forty-eight hours, the area of new epidermis obtained in some cultures was twice larger than the old fragment of skin. A few cultures were fixed and stained, and it could be seen that many cells were dividing by karyokinesis.

CULTIVATION OF ORGANS

Thyroid, Spleen, Etc.—Many cultures of glandular organs were made and grew rapidly. The cultivation of the thyroid of adult dogs was very easy. After thirty-six or forty-eight hours, long fusiform cells protruded at one or several points from the edges of the tissue. Often new polygonal cells also could be seen on the upper surface or on the edges of the thyroid. After the fifth and the sixth days, the cultures were generally in full and sometimes wild vegetation, which lasted as long as the plasmatic medium was in good condition. A great many long fusiform cells or chains of fusiform cells radiated from the tissue through the plasma. Polygonal cells were generally closer to the tissue. In a few cultures there was an abundant proliferation of cells resembling epithelial cells, while the fusiform cells were in small number.

The cultivation of suprarenal and of spleen gave also excellent results.

Kidney.—But very much more important were the results of the cultivation of the kidney. Two plasmatic media were inoculated with small fragments of a kidney of a young cat. Twelve hours later, fusiform cells were protruding from the tissue. After twenty-four hours, a great many cells had invaded the plasma all about the renal substance. One day later, the cultures vegetated wildly. On the fifth day, one of the cultures was fixed and stained with hematoxylin. We saw many karyokinetic figures in the cells which had proliferated through the plasma. A tube had begun to grow from the tissue into the medium. The cells showed a condition of great activity.

The other culture was allowed to live to the sixth day and an exceedingly active growth of the cells took place. In the morning, we observed a few tubes growing from the renal substance into the plasma. In the evening, they were very much longer and curved at their blind ends. At the beginning of the seventh day, the culture was fixed and stained. Around the renal tissue a very large number of fusiform and polygonal cells had formed. A few tubes, composed of a lumen limited by epithelial-like cells, had passed from the fragment of kidney for a distance into the plasmatic medium. They had the appearance of renal tubules.

These experiments demonstrate that adult tissues grow very easily outside of the body. Tissues like cartilage, and even like renal substance, can be caused to develop in something like normal manner under entirely new conditions.

REACTIVATION AND CULTIVATION IN SERIES

The second part of our study consisted of modifying the rate of growth of tissues by passing them into a second medium. A few six and seven day old cultures of thyroid were used for the first series of experiments. The thyroid fragments were removed from the old cultures, cut into small pieces and placed into new plasma. Eleven and twelve hours after, new cells protruded from the previously inactive parts of the thyroid substance, as well as from the newly proliferated cells. We found, indeed, that the thyroid of an adult animal had now become as active as the thyroid of an animal a few days

old. Afterward the cells invaded very quickly the new plasmatic medium. One of the cultures was fixed a little less than thirty-six hours after the passage into the new plasma and stained with hematoxylin. From one side of the old tissue there was a large mass of fusiform cells radiating through the plasma. From another point, several tubular formations had wandered far into the medium. The wall of these tubules was composed of epithelial-like cells. It seems, therefore, that the passage from one medium into another of the same kind increases the vegetative power of the thyroid cells.

In a second series of experiments, a plasmatic medium was inoculated with cells produced by the cultivation of a thyroid fragment, in order to obtain a second generation of cells. In several instances, this result was achieved. After twenty-four hours, we noted that a few cells had wandered from the old plasma into the new. In one experiment, less than four hours after the inoculation, the new plasmatic medium already contained new cells. One of these cells was fusiform and its activity was so great that we could follow under the microscope the motion of its cytoplasmic granulations and the changes of its shape. In a few minutes, one end of the cell became very large, while a long tail grew at the opposite end. Finally the cell became multipolar. Other cells which increased by division appeared at the same time in the new medium. Thirty-six hours later, the culture was fixed and stained and many active cells resembling epithelial and connective tissue cells were found to be present in the new plasma. We had, therefore, obtained a second generation of the first culture of thyroid cells.

CONCLUSION

The main results of these observations can be summarized in a few words: Adult tissues and organs of mammals can be cultivated outside of the animal body.

The cultivation of normal cells would appear to be no more difficult than the cultivation of many microbes. It remains, however, to be determined whether continuous series of cultures can be secured. This method can, therefore, be used for the study of many important problems. For instance, it may render possible the cultivation of certain micro-organisms in conjunction with living tissue cells or alone in plasmatic media. Then it will be of great value in the study of the problem of

cancer. We have already succeeded in inoculating a plasmatic medium with sarcoma of the fowl; cells appeared in the surrounding plasma after nine hours and the culture is growing actively at present. We can assume, therefore, that the perfection of the method of cultivating adult tissues of mammals outside of the body will be helpful in the exploration of unknown fields of human pathology.

Work is in progress along the lines indicated, the results of which will be published from time to time.

CULTIVATION OF SARCOMA OUTSIDE OF THE BODY

A SECOND NOTE [*]

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We have succeeded in cultivating a very malignant sarcoma outside of the body. The purpose of the experiments was to develop a general method which would permit a study of the evolution of tumor tissue under known conditions and to observe living cancer cells at every instant of their growth.

We used a fowl sarcoma that Dr. Rous has propagated from generation to generation for more than a year. Through the kindness of Dr. Rous, two chickens with actively growing tumors were placed at our disposal. Four series of cultures were made with fragments of the tumor extirpated from the animals in four different operations. The cultures started to grow after a very short latent period. While normal tissues of adult dogs and of young kittens began to develop respectively about forty-eight hours and twelve hours after inoculation of the plasmatic medium, sarcomatous tissue of the chicken showed, in some cases, evidence of activity after two and one-half hours. Fusiform cells appeared on the edge of the tissue and after five or six hours, many elongated cells and chains of cells could

be seen radiating out into the culture medium. This rate of growth approximates that observed by Burrows in sixty-hour-old chick embryos. After a very short time, the cultures reached their period of full vegetation. The growth of the tissue was extremely rapid. In a culture of the fourth set of experiments we saw, after the tenth hour, a large area of new cells surrounding the fragment of tissue. The surface area of this new growth was greater than the area of the original fragment. At the end of the first twenty-four hours the surface of the new tissue in one of the cultures was fourteen times that of the original fragment. After forty-eight hours this area might reach twenty-two times the size of the original fragment. Associated with this wide extent of new tissue was a slight decrease in the size of the old fragment. It showed that the new growth was partly built of cells wandering from the original fragment into the plasmatic medium. This wandering of cells is a phenomenon frequently noticed in the cultivation of normal tissue. But the new tissue was also composed of new cells. In a culture fixed and stained after twenty-four hours, we observed many karyokinetic figures. The new cells are morphologically different from the chick embryonal cells. They are round, fusiform or polygonal, filled completely or partially with large refractive granules. They grow in many layers in the medium and are apparently little influenced by the architecture of the fibrin net.

The nature of the plasma has a marked influence on the growth of the tumor. In seven cultures, the plasma of a normal animal was used. Only two positive results were observed. In six test cultures we employed the plasma of the animal from which the tumor had been extirpated. Six positive results were obtained.

We have endeavored to cultivate sarcomatous cells in series in order to obtain a pure culture of the more virulent elements. The second generation grew very easily.

The original tissue and the adjacent new cells of a culture
were extirpated, and the free space left by their removal
in the old culture medium was filled with new plasma.
The surface of the old medium was also covered with
new plasma. In every case the sarcomatous cells entered
immediately the new medium. In twenty-four hours
long chains of fusiform cells spreading out from the area
covered by the sarcomatous cells could be seen invading
the new medium. On the fifth day of the culture the cells
were still in full activity. Up to the present, perfect
second generation has been obtained.

These results show that sarcomatous tissue grows lux-
uriantly outside of the organism, that a second genera-
tion can be produced by the cells grown in a first culture
and that the whole process can be observed with ease at
every instant of its evolution. It is probable that the
malignant tumors of the human organism can, in a
similar manner, be caused to grow outside of the body.
The method, therefore, will be a valuable addition to our
means of studying the problem of cancer.

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HUMAN SARCOMA CULTIVATED OUTSIDE OF THE BODY

A THIRD NOTE *

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In our last note[1] we have shown that a fowl sarcoma could grow outside of the body. We expressed also the opinion that malignant tumors of the human organism could be caused to grow in the same manner. In the present note, we shall briefly describe a successful attempt to cultivate *in vitro* a sarcoma extirpated from a woman by Dr. Coley.

Through the kindness of Dr. Coley and of his assistants of the Memorial Hospital of New York, we were able to make a few cultures with fragments of a sarcomatous tumor taken from a woman 35 years old. The history of the patient given by Dr. Moore of Iowa, can be summarized as follows:

Thirteen years ago, after a traumatism, a tumor developed on the upper part of the right fibula. Last June the tumor was extirpated. It recurred soon and last September the new tumor and the fibula were removed. The growth presented then the anatomic characters of a sarcoma. It recurred again very soon and the patient was brought to the Memorial Hospital of New York.

On Oct. 27, 1910, at 4 p. m., Dr. Coley extirpated the tumor. During the operation some blood to be used as

medium was taken from the arm of the patient. At
4:30 a few fragments of sarcomatous tissue were inoculated into the plasmatic medium. Twelve cultures were
made and brought immediately to the Rockefeller Institute.

On October 28 at 8:30 a. m., that is, sixteen hours
after the inoculation, the cultures were examined. In
every culture the medium was very fragile. The main
mass of the clot had been torn away from the tissues
during the transportation of the slides. The sarcomatous fragments were held by a very thin layer of fibrin,
which was closely adherent to the cover-glass. All the
lower part of the medium was fluid. In spite of these
unfavorable conditions, fusiform cells protruded in many
points from the tissue. In several cultures, many
spindle and round cells were observed to be wandering
through the medium. Ten of the twelve cultures gave
positive results. The growth was far less active than
were the cultures of the fowl sarcoma. This was due
probably to the fact that the cells were vegetating under
difficult conditions on account of the partial liquefaction
of the medium. Nevertheless a large number of fusiform cells radiated out and wandered into the very thin
layer of plasma adherent to the glass. Their morphology could be studied very easily with an oil immersion
lens. The characters of these cells will be described in a
later publication. The purpose of the present article is
merely to show that all the details of the living cells
can be observed at every instant of their evolution.

On October 29, large fusiform cells with long tails,
round cells and a few multipolar cells were wandering
from the tissue through to the medium. The plasma
was clear and thin and the changes occurring in the
cells could be observed without difficulty. For instance,
one large fusiform cell, after having been slightly
shaken, broke loose, while we observed it. It became
immediately a small granular sphere. At 9 a. m., the
cell had the appearance of a spherical mass composed of

dense protoplasmic granulations. At 9:03 it became slightly oval. At 9:06 it was more oblong. Progressively the granulations became less densely packed in the anterior part of the cell. At 9:18 the posterior end was slightly pointed, and a clear spot appeared at the place where the granulations were less dense. At 9:20 there was a great activity among the protoplasmic granules of the posterior end. These granules were flowing into the medium and producing a short tail attached to the cell. At 9:22 the clear spot became a real nucleus with a sharp outline. A faintly opaque nucleolus appeared at the same time. At 9:25 the tail was longer. The cell also had increased very much in size. At 9:30 the posterior end developed a very long and pointed tail, while the anterior end was still blunt. The anterior end grew progressively. At 9:45 the cell had assumed the same appearance as before 9 o'clock. This observation shows how accurately the living cell can be studied in a culture.

On October 30, some of the cultures were vegetating very actively. Two of them were fixed and stained. Their examination confirmed the observations made on the living cultures. On November 1 most of the cultures were dead or had been fixed. Only one still lived on November 3.

We must conclude from this experiment that it is possible to cultivate outside of the organisms fragments of a human sarcoma in a manner similar to that of the animal sarcoma previously described. Therefore we will probably be able to study *in vitro* the growth of the various human malignant as well as benign tumors and to follow all the morphologic characters and changes of the cancerous and other cells during life.

METASTASIS AND TUMOR IMMUNITY

OBSERVATIONS WITH A TRANSMISSIBLE AVIAN NEOPLASM*

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In a previous article,[1] I have reported some observations on a sarcoma of the chicken, a typical neoplasm, which, unlike the avian tumors already studied, has shown itself transplantable from fowl to fowl. At this writing the sarcoma has developed an extraordinary malignancy and gives wide-spread metastasis. Work with it has brought out facts which bear largely on the general problem of tumor metastasis and tumor immunity.

The sarcoma has passed through six generations of inoculated fowls during the last thirteen months. It is of spindle-celled variety and has throughout remained true to type. In the degenerated portions true mucin may be found. The specificity which at first limited its successful transmission to fowls of the same pure-bred stock in which the growth arose has been to some extent overcome. It now grows in about 85 per cent. of these pure-bred fowls—light, barred Plymouth Rocks—and in an occasional individual that shows by its plumage the slight admixture of some darker strain; but it grows only in Plymouth Rocks. Retrogressions of the developed tumor are rare.

The increase in the malignancy of the sarcoma followed the use of young hosts. Prompt invasion and metastasis are now the rule. Grafts removed and exam-

ined three days after implantation are found vascular-
ized, and the sarcoma cells have already pushed into the
host's tissues well beyond the boundary of the intro-
duced bit. A fragment 1 to 2 mm. in diameter, placed
with a trochar in the breast muscle, may give rise within
three weeks to a mass measuring 13.5 by 7 by 5 cm.
From clavicle to lower sternum the muscle fibers are
almost completely replaced by tumor. The host rapidly
emaciates, becomes cold, weak and somnolent and shortly
dies.

Metastasis takes place through the blood-stream and
much more rarely through the lymphatics. The lungs
are often almost completely replaced by coalescing
growths, and less frequently the liver and kidneys show
nodules. The serous membranes may be penetrated and
a wide-spread peritoneal dissemination follow. Intra-
peritoneal growths may cause intestinal occlusion, or
may unite into a common mass viscera that lie far apart.
In general the secondary tumors spare or affect the same
organs as in mammals, the spleen, for example, enjoy-
ing an almost complete immunity as compared with the
lungs, liver and kidneys. The reason for this freedom
of the spleen from tumor metastasis is not apparent.

Metastases appear in the heart with great constancy,
a feature not seen in mammals. Both sides of it are
involved about equally often, and the origin may be
almost completely replaced by tumor tissue before the
host dies. In such cases it is found greatly enlarged,
pale, stiff, and roughened by growths that have broken
through on its surface. The pericardium is thickened
with contact metastases.

Metastases develop best when the primary tumor
grows rather slowly. When it grows rapidly the host dies
before the secondary nodules have had time to reach a
large size. In such animals great numbers of minute
metastases are found, but apparently a sufficient interval
for their development has not elapsed. The growth-rate
of the primary tumor has increased without a correspond-
ing increase in the rapidity of metastasis formation.

This is doubtless because some of the more or less mechanical processes concerned in the invasion of capillaries, breaking off of cells, their transportation, lodgment and vascularization by the host tissue are not to be hastened beyond a certain limit by mere increased growth-rate of the neoplasm.

A study of grafts of the sarcoma removed at short intervals from susceptible fowls, and fowls with a natural or enhanced resistance, has shown that this resistance does not depend on the absence of a supporting and vascularizing reaction for the graft—a phenomenon held by many to be of primary importance in tumor immunity. It is true that an absence of such a supporting reaction is not infrequently responsible for the death of grafts in the resistant animal, especially when they have been placed in a site where connective tissue is nearly wanting; but in by far the greater number of cases the graft undergoes vascularization. Its success is nevertheless of short duration. In the first few days after inoculation, while it is growing and seems healthy, there occurs a rapid accumulation of small round cells, (lymphocytes) first about the near-by blood-vessels, then extending around and into the tumor graft. A week from the time of its implantation this is so inclosed by small round cells (and to a much less degree by large mononuclears, plasma-cells and fibroblasts) that on a casual glance one would think it part of a lymph-gland. At this time it is, as a rule, rapidly degenerating, although well vascularized. Exceptionally it does not die but continues to proliferate, despite the indications of resistance by the host, and eventually establishes itself. In susceptible fowls one finds at the edges of the growing tumor a less marked reaction of the sort described. There are traces of it about the original neoplasm. It is not called forth by material killed by heat (60 C. for thirty-five minutes) or repeated freezing and thawing.

Immunity in the case of this chicken sarcoma does not then depend primarily on the absence from the host

tissues of a supporting reaction and vascularization for the graft. There are indications in the literature that this phenomenon, so obvious in rats and mice, is here also secondary to less understood processes. Burgess[2] examining grafts removed from mice racially insusceptible to the tumor inoculated. has found them vascularized and growing for a short period, then surrounded by granulation tissue and degenerating. Da Fano[3] after a careful histologic study of tumor grafts in mice, and of the sparse cellular reaction about them, has come to the conclusion that lymphocytes are in some way connected with tumor immunity. About the growing tumors of man lymphocytes are not infrequently seen in considerable numbers.

The conclusion seems justified that resistance to tumor growth can no longer be considered to depend *primarily* on a failure of the host to provide a stroma and vascularization for the neoplastic cells. Both may be provided by the resistant host, and yet the tumor dies. Whether the lymphocytes which accumulate about it, or some factor unknown, is responsible for this result, has yet to be investigated.

The chicken sarcoma has recently been cultivated *in vitro* by Drs. Carrel and Burrows.[4]

Sixty-Sixth Street and Avenue A.

2. Burgess, A. M.: Jour. Med. Research, 1909, xxi, 575.
3. Da Fano, C.; Ztschr. f. Immunitätsforsch, 1910, v, 1.
4. Carrel, A., and Burrows, M. J.: Cultivation of Sarcoma Outside of the Body, THE JOURNAL A. M. A., Oct. 29, 1910, lv, 1554.

THE TREATMENT OF WOUNDS

A FIRST ARTICLE *

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In the actual condition of therapeutics, aseptic wounds
generally heal in a few days. The more ambitious
dreams of the surgeons of the pre-Listerian era have been
fulfilled. Nevertheless, we have no right to believe that
the treatment of wounds has reached its ultimate perfec-
tion. We must investigate whether or not it is possible
to advance farther. In the treatment of wounds, we con-
tent ourselves by protecting the tissues against infection.
and we leave to Nature the care of cicatrization. Would
it not be feasible to act on the processes of reparation
themselves and to activate them? The wounds which
now heal in a few days could possibly be caused to heal
in a few hours. The treatment of fractures would also
be simplified. The development of methods for the
stimulation of the growth of epithelial cells, for the inhi-
bition or the activation of the proliferation of connective
tissue, for the artificial production of osteogenesis, etc..
would greatly improve the therapeutics of the ulcerations
of the skin and of the lesions of peripheral nerves, bones
and many other tissues or organs. This new evolution of
surgery depends on the discovery, partial at least, of
the laws of redintegration of tissues of mammals. Cica-
trization and regeneration are the expression of the
power to persist in its form with which all organisms are
endowed. We are deeply ignorant of the nature of this
function of redintegration. It is, as is the function of
nutrition, a fundamental property of living matter. To
know its nature is as impossible as to know the nature of
life. Besides this knowledge would be useless. From a
metaphysic standpoint it would be interesting to discover
why a wound heals. But from a scientific standpoint. it

is infinitely more important to know *how* it heals, because it would then be possible to find what stimuli start the complex mechanisms of the regeneration of the tissues. Therefore, the physiologic phenomena of cicatrization must be investigated. It is true that the power of redintegration escapes our methods of research. But the physico-chemical processes which this power, as a directing idea, coordinates and harmonizes in view of the morphologic reparation, can be brought into the field of experiment. We must, therefore, analyze the mechanisms which are instrumental in the cicatrization of a wound, the factors which modify their functions, the stimuli by which they are started, and the causes of their reciprocal cooperation to the common work. Perhaps it will become possible to use some of these agents for the artificial activation of the regeneration of tissues and the treatment of wounds.

MECHANISMS OF THE REPARATION OF A CUTANEOUS WOUND

Since many centuries all surgeons know the anatomic processes of the cicatrization of a wound. On the open surface, granulations appear, and, by their contraction, bring closer to each other the edges of the epidermis. Then the epithelial cells wander on the granulous tissue and a new epidermis is formed. These phenomena can be divided into four periods: quiescent period, period of granulous retraction, period of epidermization and cicatricial period.

The experiments on which this article is based were performed chiefly on dogs. The cicatrization of wounds obtained by resection of a flap of skin was observed. The resected flap was of geometrical form, rectangular, trapezoidal or circular. In order that the edges of the old epidermis might be easily seen, I used black animals or I stained the edges of the wound with India ink. It was then possible always to distinguish the new from the old epidermis, and to follow accurately the varia-tions of the dimensions. The dressing consisted of tal-cum powder and gauze or warm paraffin. The wounds were kept as nearly aseptic as possible. When they became infected the results were discarded.

1. *Quiescent Period.*—The quiescent period extends from the time of the resection to the time of the beginning of the granulous retraction. During the first days the dimensions of the wound do not vary. If we repre-

sent graphically by a tracing the time of healing the successive distances between two points A and B taken on the opposite sides of a rectangular wound, the tracing during the quiescent period is horizontal. Suddenly it inclines downward. It is the beginning of the granulous retraction. Often the immobility of the edges of the wound during the quiescent period ceases rather suddenly; there is no period of transition and the active period of reparation starts immediately. The main characteristic of the quiescent period is the great variability of its duration. In some cases it lasts only one or two days, while in others it lasts four or five days.

2. *Period of Granulous Retraction.*—At the end of the quiescent period the edges of the wound begin to advance toward each other. The tracing of the consecutive distances between the points A and B. taken on the opposite sides of a rectangular wound, shows a sudden inclination downward. Progressively, the inclination of the curve diminishes and, after a few days, it is almost horizontal. The reduction in size of the wound is very active during the first days of the period of granulous retraction. Then it becomes progressively slower until it comes to a standstill. This fact was observed long ago. It was believed that the activity of the granulations depended on their age, while it depends really on the dimensions of the wound.

By measuring the rate of reparation of a rectangular wound, I found that it diminishes progressively from the beginning to the end of the period of granulous retraction. The rate when the wound is 60 or 70 mm. is about 9 or 10 mm. for twenty-four hours. When the wound is one of 40 mm. the rate is about 3 mm. When the dimensions of the wound are only 20 mm. the rate becomes very slow. In all wounds the rate becomes about zero when the edges have reached a distance of about 10 or 15 mm. from each other. It is therefore certain that these differences in the rate of reparation are functions of the size of the wound.

By observing on the same animals large and small wounds, I could see during the same period the larger wound diminishing with a much greater speed than the smaller wound. For instance, two rectangular wounds (Experiment 176) were made on the same animal. The transverse dimension of the one was 66 mm. and of the

other 26 mm. During the first forty-eight hours of the
period of granulous retraction, the larger wound
diminished 20 mm. and the smaller 4 mm. On trape-
zoidal wounds, it was observed also that the reduction
in size of the smaller side is very much slower than the
reduction undergone by the larger side. On circular
wounds, made on the same animals with cutting tubes
1 and 2 cm. in diameter, the same phenomena were
observed. Many other experiments have been performed.
It is certain that the *rate of reparation* of the granulous
period is *directly proportional to the size of the wound:*
that is, to the effort to be accomplished in order to bring
about the redintegration of the parts. It must be
noticed that during the period of granulous retraction,
the redintegration of the skin of mammals follows the
law discovered by Spallanzani on the salamander. If
the tail of a fish or salamander is cut off near its base,
the new part grows faster than when the tail is cut off
nearer the tip. The new part which arises from the
basal cut grows more rapidly at first and more slowly
later. The rate of regeneration is proportional to the
importance of the work to be done. It is remarkable
that, on mammals, the reparation of the skin, which is
brought about by a very different mechanism, obeys the
same general law.

The period of granulous retraction plays a very
important rôle in the healing of the middle-sized and
the large wounds. Thus, a wound of 60 or 70 mm. can
be reduced to one-third and one-fourth of its primitive
size. A wound of 30 mm. can be reduced to one-half
its size. The importance of the granulous period is less
for the small wounds. The effort of the granulations on
·a wound of about 15 mm. reduces its dimensions to
three-quarters or even less of its original size.

The end of the granulous period corresponds to the
beginning of the epithelial wandering from the edges
of the wound. For a wound of about 30 or 40 mm. the
retraction becomes exceedingly slow and even stops com-
pletely when the distance between the edges has been
reduced to 10 or 15 mm. When the wound is larger,
the retraction stops often when its dimensions are still
20 or 25 mm. In that case the epithelial wandering is
very slow and often the result is an ulcer.

It seems that the epithelial wandering on the surface
of the granulations stops immediately their retraction.

The epidermization not only coincides with the end of the period of granulous retraction, but it causes it. In the corner of a granulating rectangular wound I deposited a small square graft of skin. After a few days the graft was found surrounded by the normal skin of the edge and the wound had assumed again a perfectly rectangular appearance. This shows that the retraction stopped at the level of the graft, while it still went on in the other parts of the wound. In another case, the epidermization of the upper part of a large square wound was stimulated by a graft, while the lower part remained without epithelium. It was then observed that the distance between the India-ink-stained edges of the old epidermis was still diminishing in the lower part, while it increased in the upper part. The shape of the square wound became trapezoidal. Sometime after complete epidermization it became square again. It is, therefore, certain that the epidermization inhibits the retractive function of the granulations. When the epidermization takes place early, the scar is large and thin. When the epidermization is late, the granulations undergo a stronger retraction and the scar is thick and comparatively smaller.

The function of the granulous period is also to prepare the surface of the wound for the wandering of the epithelial cells. But it seems probable that its main rôle is to bring the edges of the wound to a certain distance—about 10 or 15 mm. in the dog. It is shown by the fact that if the wound is only 10 mm. wide, practically no retraction occurs. It does not occur because it would be useless, since at the distance of 10 mm. the next mechanism of the reparation, that is, the epithelial wandering, can take place easily, as will be shown later.

3. *Period of Epidermization.*—On a rectangular wound, the edges of which are stained with India ink, it is easy to detect the beginning of the period of epidermization. The new epithelium spreads at first very slowly on the surface of the granulations. It is difficult to locate exactly the free edge of the new epidermis. Nevertheless, by using paraffin dressing, it is possible to see with certainty in a few cases the edge of the wandering epithelium. The new epidermis is exceedingly delicate and a great many external factors interfere with its growth. The best medium for its growth is certainly coagulated fibrin, which can be obtained by using as a

dressing paraffin of a certain consistency and melting-point.

The time of the beginning of epidermization does not depend on the age of the wound but on its dimensions. If the wound is large, the epidermization is tardy. It occurs very much earlier when the wound is smaller.

By measuring the distance between two points taken on the free edge of the new epidermis of a rectangular wound, it was found that the growing of the epithelium is exceedingly slow if the distance is more than 12 or 15 mm. But if the edges are located less than 10 mm. from each other, the epithelium wanders more quickly on the granulations. When the free edges 'of the epithelium are closer, the rate of cicatrization is very much faster. I found, in one case, a rate of 2.5 mm. for a distance of 5 mm. The curve representing the positions of the two points runs at first almost horizontal and progressively inclines itself downward with an accelerated rate.

When at the end of the granulous retraction of a large wound, the edges of the old epidermis are still at a distance of 20 or 25 mm. the new epidermis cannot spread on the granulations and the cicatrization of the wound comes to a standstill.

It seems that the time of the epidermization and its rate depends mainly on the dimensions of the wound. This point has been ascertained by several other sets of experiments.

By observing trapezoidal wounds it was found that the smaller side about 8 mm. wide was completely epidermized while the larger side about 20 mm. wide presented an epithelial band of about 2 mm. along the edges of the old epidermis. On irregular wounds the epidermization begins always on the points where the edges are closer to each other. In lozenge-shaped wounds the epidermization begins in the acute angles and the wound becomes an ellipse. On several kinds of trapezoidal wounds it was always found that the epidermization begins sooner and spreads more quickly between the points which are separated by the shortest distance.

Therefore, it appears that the law of reparation by epidermization is absolutely different from the law of separation by granulous retraction. *The rate of the epidermization is inversely proportional to the dimensions of the wound.* It is very slow when the distance

between the edges of the wound is more than 10 or 15
mm. The maximal activity of the epidermization seems
to take place when the cicatrization is nearly complete,
and when the edges of the new epithelium are very close
to each other.

4. *Cicatricial Period.*—The dimensions of the scar
can easily be measured when the edges of the old epi-
dermis are stained with India ink, or when the animal
is black. It was found that the scar of a large wound
is comparatively smaller than that of a small wound.
On the same animal, two wounds of 66 mm. and of 26
mm. were observed. The 66 mm. wound gave a scar of
22 mm. and the 26 mm. wound a scar of 13 mm. The
scar of the large wound was only one-third the size of
the wound, while the scar of the small wound was one-
half the size of the wound. If the wound is still smaller,
10 or 12 mm., the scar is almost the same size as the
wound. This is the natural result of the law of granu-
lous retraction.

The evolution of the scar is very slow and the cica-
tricial period of a wound very long. As soon as the
epidermization is completed, the distance between the
points A and B of the edges of the old epidermis grows
greater. The tracing shows a slight movement upward
of the line representing the different values of the dis-
tance A and B. The points A and B have a tendency
to go back to their former position. This progressive
enlargement of the scar lasts for a long time and its
result should be a complete redintegration.

The mechanisms which are instrumental in the cica-
trization of a wound are coordinated in such a way that
the reparation is continuous and progressive. Never-
theless, the reparation presents phases of maximum
and minimum activity during which the rate is higher
or lower. During the quiescent period, the end of the
period of granulous retraction and the beginning of the
period of epidermization, the rate of the reparation is
slow. It is maximum at the beginning of the period of
granulous retraction and at the end of the period of
epidermization. The two mechanisms are adapted to
the healing of small and middle-sized wounds, the width
of which is not over 40 mm. In a wound 30 or 40 mm.
in width or smaller, the retraction of the granulations
is very efficient, since it can quickly bring the edges to
a distance of 10 or 15 mm. This distance is very favor-

able to the epidermization. Therefore, at the same time when the rate of reparation by granulation becomes very slow, the epidermization starts and the reparation goes on without interruption, although by a different mechanism. But if the wound is larger, 60 or 70 mm., the retraction of the granulations cannot bring the edges to the minimum distance. They remain at a distance of about 20 mm. and the reparation comes to a standstill because the epidermization cannot take place easily under these conditions. The mechanisms are very efficient for the healing of the injuries to which the animals are exposed in their every-day life. But they do not work as satisfactorily for the larger wounds.

CHEMO-IMMUNOLOGICAL STUDIES ON LOCALIZED INFECTIONS.[1]

FIRST PAPER: ACTION ON THE PNEUMOCOCCUS AND ITS EXPERI-
MENTAL INFECTIONS OF COMBINED SODIUM OLEATE AND
ANTIPNEUMOCOCCUS SERUM.

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WITH AN INTRODUCTION BY

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INTRODUCTION.

The phenomenon of recovery from any local bacterial infection has not been fully explained, and it is not wholly accounted for by the several activities of blood serum and phagocytes, which have often been viewed as gradually overcoming and removing the offending bacterial agents. Indeed, this restricted view has never been fairly established either by direct observation upon affected human beings or animals, or through experiment; and it leaves out of account the effects of certain definite chemical substances, other than antibodies, which are always present in a focus in which tissues and cells are disintegrating. That some of these chemical substances are injurious to bacteria and even to somatic cells we now know, and it has been found possible to define more precisely the chemical nature of part, at least, of the injurious chemicals.

It has long been known that certain somatic cells yield upon extraction bactericidal substances that are neither complements nor amboceptors. Thus the long series of investigations, beginning with those of Buchner[2] and Denys,[3] including those of Hahn,[4] Schat-

[1] Received for publication, August 1, 1910.
[2] *Arch. f. Hyg.,* 1890, x, 84.
[3] *La Cellule,* 1893, ix, 335; 1894, x, 5.
[4] *Arch. f. Hyg.,* 1895, xxv, 105.

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tenfroh,[5] van der Velde,[6] Bail,[7] Moxter,[8] and Löwit,[9] and terminating with those of Pettersson[10] and Zinsser,[11] have eventuated in proving that the substances extracted or expressed from leucocytes are capable of destroying bacteria of a wide series ranging from the pyogenic cocci, on the one hand, to the bacilli typhosus, coli, and anthracis, on the other. The distinguishing characteristics of these intracellular bactericidal substances are their thermostability and capacity to act independently of the presence of alkaline salts in the medium.

These extractive substances, obtained either directly from cells or after autolysis, have been shown by Conradi,[12] Korschun and Morgenroth,[13] Levaditi,[14] and Noguchi,[15] to be not only bactericidal, but often hemolytic as well, and to differ still further from the thermolabile antibodies in being alcohol-soluble. Very recently Noguchi[16] has determined that the activity of these extracts depends largely, if not wholly, upon their content in certain higher unsaturated fatty acids or their alkaline soaps. That soaps occur in inflammatory foci has been shown by Klotz;[17] and the origin of the fatty acids and soaps is readily accounted for since Achalme[18] has proved the presence of lipase in such foci. That neutral fat and even the higher phosphorized fats or lecithin complexes occur in disintegrating tissue and exudate is well known; and under the influence of the lipase, which Bergel[19] believes to be especially abundant in the mononuclear leucocytes or lymphocytes,

[5] *Arch. f. Hyg.*, 1897, xxxi, 1; 1899, xxxv, 135.

[6] *Centralbl. f. Bakteriol., 1te Abt.*, 1898, xxiii, 692.

[7] *Arch. f. Hyg.*, 1897, xxx, 348.

[8] *Deutsch. med. Wchnschr.*, 1899, xxv, 687.

[9] *Beitr. z. path. Anat. u. z. allg. Path.*, 1897, xxii, 172; *Centralbl. f. Bakteriol., 1te Abt.*, 1898, xxiii, 1025.

[10] *Centralbl. f. Bakteriol., Orig.*, 1905, xxxix, 423, 613; 1908, xlvi, 405.

[11] *Jour. Med. Research*, 1910, xxii, 397.

[12] *Beitr. z. chem. Phys. u. Path.*, 1902, i, 193.

[13] *Berl. klin. Wchnschr.*, 1902, xxxix, 870.

[14] *Ann. de l'Inst. Pasteur*, 1903, xvii, 187.

[15] *Biochem. Ztschr.*, 1907, vi, 327.

[16] *Loc. cit.*

[17] *Jour. Exper. Med.*, 1905, vii, 633.

[18] *Compt. rend. Soc. de biol.*, 1899, li, 568.

[19] *München. med. Wchnschr.*, 1909, lvi, 64; 1910, lvii, 1683.

these fats are decomposed and their fatty acids liberated. Certain tissues which have undergone autolysis *intra vitam* yield an alcoholic extract of high hemolytic activity, as has been shown for the liver in acute yellow atrophy, toluylendiamin and phosphorus poisoning, by Joannovics and Pick,[20] Ehrmann and Stern,[21] and Jacoby,[22] and this unusual potency is ascribed to the presence of free, higher unsaturated fatty acids which are derived from cleavage of the lecithin-like complexes. Dr. Lamar has extracted relatively large quantities of such lecithin-like bodies or lipoids and fatty acids from the human lung, the seat of resolving lobar pneumonia.

The quantity of bactericidal substances extractable from leucocytes *in vitro* must be a small measure of those which may be liberated from leucocytes in an inflammatory focus. These extractable substances represent merely the preformed bodies and are minimal in amount. While it is probable, it has yet to be proved, that they consist of soaps. The leucocytes in an inflammatory focus, on the other hand, suffer successive death and disintegration either through the action of bacterial poisons—leucocidins—or because of lack of nutriment, and then yield through autolysis and lipolysis the fatty complexes out of which fatty acids and soaps are produced. The quantity of bactericidal and hemolytic substances yielded by cells in process of autolysis is relatively considerable. The disintegration of leucocytes and tissues which results from a local bacterial infection can, therefore, be viewed as a process that is not entirely to the advantage of the parasitic agent, but is also of use to the economy in assisting to overcome the bacteria, since the cells brought to death and disintegration by the parasites yield certain definite chemical substances that themselves exert a destructive action upon the infecting bacteria.

Turning now to the subject of the pneumococcus, with which this paper deals, and considering the typical form of pneumococcus inflammation presented by lobar pneumonia, it should first be stated that the pneumococci present in the resolving exudate:

[20] *Ztschr. f. exper. Path. u. Therap.,* 1909, vii, 185; *Berl. klin. Wchnschr.,* 1910, xlvii, 928.

[21] *Berl. klin. Wchnschr.,* 1910, xlvii, 282.

[22] *Berl. klin. Wchnschr.,* 1910, xlvii, 677.

undergo a progressive diminution, both in number and in virulence, as the process of resolution proceeds.   This decrease in the number of pneumococci is not produced by phagocytosis, which occurs, as a rule, but slightly; and test tube experiments have shown that the pneumococcus is little, or not at all, subject to serum-lysis; hence some other mechanism must be invoked to explain their removal. Many years ago Welch[23] observed that the resolution of the pneumococcic exudate was accompanied by the lysis of the pneumococci within their capsules, and that all stages of the chromatolysis of the organisms, up to complete disappearance, could be followed under the microscope.   What the nature of the agency is that brings about this form of solution of the pneumococci within their capsules has not been determined.   Now it becomes significant, as Dr. Lamar's investigation shows, that pneumococci which have been exposed to the action of weak solutions of sodium oleate become so altered in texture that, while still viable in cultures, they are subject to lysis or solution by means of the body fluids and particularly by means of an antipneumococcus serum *in vitro* or *intra vitam.*

Such an assumption as that the alteration in virulence and number suffered by pneumococci during resolution of a lobar pneumonic exudate depends in part upon the action of the fatty acids and soaps of the exudate, would, in view of the facts presented, have some justification; and this assumption would be further strengthened by the additional fact ascertained by Dr. Lamar, that the viable, soap-treated pneumococci have had their virulence diminished by the treatment.

On the other hand, an obstacle immediately appears in the way of this highly satisfactory explanation.   Noguchi[24] and von Liebermann[25] have separately noted that hemolysis and bacteriolysis by the unsaturated fatty acid soaps are inhibited by the protein of the serum; and the question, therefore, arises whether the acids and soaps produced in the resolving exudate could come to act injuriously upon the bacteria and in this case upon the pneumococci. No definitive reply can be made at the moment to this question,

[23] *Johns Hopkins Hosp. Bull.,* 1890, i, 73.

[24] *Loc. cit.*

[25] *Biochem. Ztschr.,* 1907, iv, 25.

since we are still too ignorant of the interaction of chemical substances within a resolving exudate. But we may still consider factors that possibly suffice to overcome this initial impediment to the action of the soaps, among which are the proximity of the bacteria to the nascent fatty acids and soaps and the natural occurrence within the exudate of chemical bodies that have the effect of removing the protein inhibition similar to that described for boric acid in the experiments of Dr. Lamar.

The complexity of the defensive processes invoked against bacteria is further emphasized in a suggestive way by the striking part taken by the antipneumococcus serum in the experiments. The action of combined sodium oleate and immune serum upon the lysis of the pneumococci and upon infection with that organism indicates a highly important property of immune serum. The destructive and protective effects described in connection with the combination are probably not simply those of summation of two independent injurious agencies, but may well be the expression of a new and specially acquired property of the antiserum, since the normal serum fails to produce perfect and complete lysis of the soaped pneumococci. Moreover, it has been shown clearly, especially by Neufeld[26] and his associates, that, at the time of the appearance of the crisis in pneumonia, antibodies are demonstrable within the blood; thus, it would appear that the conditions afforded by the inflammatory exudate for the interaction of the soaps formed within the exudate and the bacteria there present may come to be favorable to the destruction of the pneumococci.

We entertain the opinion that our knowledge of the phenomena of local destruction of infecting bacteria may be promoted by a more comprehensive study of the chemistry of the process and by declining to confine attention to the antibodies and phagocytes exclusively. The purpose of the present paper, and of those which it is expected will follow, is to attempt to define the part played in destroying bacteria by the more definite chemical bodies occurring within local inflammatory foci, especially those undergoing resolution, and to put to a practical test, in promoting recovery from experimental infections, the facts ascertained in the course of the investigation.

[26] *Arb. a. d. k. Gsndhtsamte.*, 1910, xxxiv, 166.

ACTION ON THE PNEUMOCOCCUS AND ITS EXPERIMENTAL INFECTIONS OF COMBINED SODIUM OLEATE AND ANTIPNEUMOCOCCUS SERUM.

CONDITIONS OF PNEUMOCOCCUS INFECTION.

The state of our knowledge of the conditions of pneumococcus infection will be indicated by the brief review of the literature that is to follow. The effects of the entrance of bacteria into an animal body are considered to depend chiefly upon the susceptibility of the animal and the virulence of the invading bacteria. These expressions are obviously merely relative and bear a reciprocal relationship, yet at the extremes the states of susceptibility and virulence are well defined and easily appreciated. The conditions are well illustrated by the manner of reaction of different animals to the pneumococcus. Certain animals, under normal conditions, represent one extreme of entire insusceptibility to infection with the pneumococcus, and others present the opposite extreme of high susceptibility to its effects. Thus, the pigeon is entirely resistant to infection, while the goat, dog, sheep, guinea pig, rat, mouse, and rabbit, as well as man, must be ranked among the susceptible animals. The reactions of the susceptible animals vary. There may be only a slight local inflammation, or a severe local inflammation with or without septicemia, or an immediate fatal septicemia following a slight local reaction. Experimental evidence exists to the effect that the susceptibility of the dog to experimental infection with the pneumococcus is similar to that of man to spontaneous infection. That is, in man and the dog, pneumococcus infections are usually of the nature of severe local reactions, which only exceptionally are attended by true septicemia, as distinguished from the early transitory bacteremia. But in man, as in susceptible lower animals, the effects depend, in considerable part, upon the degree of virulence of the pneumococci. The pneumococci which thrive saprophytically in the buccal cavity of human beings are apparently, as a rule, harmless, and when cultivated from this source the organism has little pathogenicity, sometimes none, for the highly susceptible mouse and rabbit. However, when the pneumococcus is associated with local inflammations in human beings, of which

many varieties occur, both as regards situation and severity, the pneumococci cultivated from the lesions exhibit all degrees of pathogenicity for these very susceptible animals. The organisms obtained from most of the local inflammations are not highly pathogenic, but those obtained from pneumonic lesions are sometimes, although not invariably, of high potency.

The basis of these differences in susceptibility of animals and in virulence of different strains of pneumococci has been sought from the time of the earliest work by the pupils of Metchnikoff to that of the recent studies of Hektoen[27] and his pupils, especially in relation to phagocytosis. Tchistovitch[28] concluded from his experiments that the state of refractoriness or susceptibility to infection depended wholly upon whether the leucocytes of the animals did or did not ingest and destroy the pneumococci. Mennes[29] further noted that an animal artificially immunized against the pneumococcus resisted infection because its leucocytes exercised increased power to take up the organisms. Moreover, his studies of the virulence of different strains of the diplococcus led him to conclusions similar to those arrived at by Marchand in his studies of the streptococcus, namely, that the avirulent pneumococci are readily taken up by phagocytes, while the virulent are not. He therefore concluded that a pneumococcus produces infection because it possesses the power to resist destruction by leucocytes, a view which has been confirmed and accepted by many later workers. Recently Strouse[30] has added that virulence depends in part upon the ability of the pneumococcus to grow in the body of the animal, and not wholly upon its resistance to phagocytosis.

Much attention has been given to the study of the causes that underlie the resistance to phagocytosis shown by virulent pneumococci. Marchand[31] ascertained that the killing of the organisms by heat and the treatment of them with various chemical substances did not render the previously virulent organisms subject to phagocytosis, from which he concluded that the determining factor was

[27] *Jour. Infect. Dis.,* 1906, iii, 446, 731; 1907, iv, 285; 1908, v, 273.
[28] *Ann. de l'Inst. Pasteur,* 1890, v, 285.
[29] *Ztschr. f. Hyg.,* 1897, xxv, 413.
[30] *Jour. Exper. Med.,* 1909, xi, 743.
[31] *Arch. de méd. expér. et d'anat. path.,* 1898, x, 253.

some physical state, and not the chemical composition, of the pneumococci. Recently, however, Rosenow[32] has attributed the resistance to phagocytosis offered by virulent pneumococci to a substance called by him "virulin," which he believes he has shown to be present in the virulent organisms not subject to phagocytosis and to be absent in the avirulent ones; and, moreover, that the substance may be separated from the organisms containing it through autolysis. Similarly, Tchistovitch and Jourevitch[33] have stated that virulent pneumococci may be rendered subject to phagocytosis by merely washing them with salt solution which removes a substance called by them "antiphagine," upon which they believe the resistance to phagocytosis depends.

Studies of the action of immune or antipneumococcus serum have succeeded in establishing its biological properties and in determining that its protective value against infection is slight. Such immune sera exert only a slight agglutinating action upon the pneumococci and are devoid, or nearly so, of antitoxic and bactericidal action. They differ from the corresponding normal sera chiefly in their content in bacteriotropin or opsonin, through which they promote phagocytosis to a greater degree than normal serum does. It is true that when immune sera are injected into the body of certain susceptible animals in advance of the inoculation of the pneumococci, infection is sometimes prevented, which, according to Neufeld[34] and his pupils, is the result solely of the action of the specific bacteriotropin. Yet these immune sera have proven powerless to cure an established pneumococcus infection. To this statement there is, possibly, one exception. Römer[35] believes that the antipneumococcus serum is effective in the treatment of corneal ulcers caused by the pneumococcus.

EXPERIMENTAL OBSERVATIONS.

With our present knowledge, we must view the power of an antipneumococcus serum to promote phagocytosis as the index of

[32] *Jour. Infect. Dis.*, 1907, iv, 285.
[33] *Compt. rend. Soc. de biol.*, 1908, lxiv, 1095.
[34] *Ztschr. f. Immunitätsforsch.*, 1909, iii, 159.
[35] Serumtherapie der Pneumokokkeninfektion der menschlichen Cornea, Wiesbaden, 1909.

its therapeutic activity, and we must also view the property of spontaneous phagocytosis of the pneumococci, which certain animals exhibit, as the determining cause of their refractoriness to infection. The only known exception to this is the pigeon, whose refractoriness Strouse[36] believes to be due to its normally high temperature. These considerations do not exclude the probability that there may be still other and undetermined factors concerned in the prevention of infection by the pneumococcus.

Sodium Oleate and Lysis of the Pneumococcus.—Although antipneumococcus serum possesses in itself neither bactericidal nor bacteriolytic power, we have found that when it acts in conjunction with certain chemical substances, it comes to exert both of these actions. The chemical substances which we have studied especially are the alkaline soaps of oleic acid. That soaps are bactericidal for certain bacteria, namely, the bacilli of typhoid fever, anthrax, and dysentery, has previously been shown by Noguchi.[37] We have noted that the oleates of sodium, ammonium, and neurin are bactericidal for the pneumococcus also, and that while the effects are, in general, qualitatively alike, the sodium soap is perhaps the most, and the neurin soap the least, active of the series. In the experiments to be described, sodium oleate was employed exclusively.

In the experiments virulent pneumococci have been subjected to several different concentrations of sodium oleate, alone and in conjunction with normal and immune sera. In the first place, in order to determine the effects upon the pneumococci of the soap solutions alone, they have been added to broth cultures, and microscopical studies have been made for morphological and tinctorial changes; and, in addition, tests were made to ascertain the viability on artificial culture medium of the treated diplococci, as well as their degree of virulence for rats and mice.

The virulence of the cultures employed was maintained at a high point by daily passage through mice. The greater part of our studies was made with two cultures, designated "74" and "N.1," which throughout the period of the experiments were fatal in doses of 0.000001 cubic centimeter for a full grown mouse, and

[36] *Loc. cit.*
[37] *Biochem. Ztschr.*, 1907, vi, 327.

0.00001 cubic centimeter for a grown rat.   In each experiment a culture in broth eighteen to twenty-four hours old, made directly from the heart's blood of a "passage" mouse was employed, and in each, control animals were employed to guard against any sudden change in the pathogenicity.

When sodium oleate is added to a broth culture of the pneumococcus in strengths of 1 to 0.5 per cent. of the volume, the diplococci are killed within fifteen to thirty minutes.   In hanging-drop and film preparations the diplococci are seen to disintegrate slowly without swelling.   To the naked eye the mixture clears only slightly and becomes viscid.   This conversion of the disintegrated cocci and soap into mucin-like material is more strikingly apparent when the number of cocci in the mixture is increased, as by centrifugalizing the culture and resuspending the sedimented diplococci in broth or salt solution to one-fourth of the original volume of culture.   Then nearly the whole of the mixture becomes converted into a thin translucent jelly which persists unchanged for two days.   In this respect the action of sodium oleate differs strikingly from that of bile which causes complete solution of the diplococci and renders the suspension perfectly clear, transparent, and limpid.

Greater dilutions of the sodium oleate produce less profound changes in the pneumococci, and when the dilution of the soap is greater than 1 to 1,000, the pneumococci are not killed after one hour's contact.   When such mixtures of soap and culture are centrifugalized at a high rate of speed the pneumococci collect in the bottom of the tube in the form of a whitish coherent slimy mass, which upon being resuspended in salt solution may be readily broken up into a fine suspension resembling the original culture.

When pneumococci which have been treated with sodium oleate in the way described and suspended in salt solution are allowed to undergo autolysis, it is found that they disintegrate more quickly than do normal pneumococci of the same origin suspended also in salt solution.   While the former undergo complete disintegration in a few hours so as to leave the fluid clear, the latter are incompletely disintegrated after a lapse of two days.

If the strength of the sodium oleate is still further reduced to

dilutions of 1 to 10,000, 15,000, and 20,000, the exposed cocci are not altered in morphological appearance, staining properties, or ability to grow upon artificial culture medium, yet they still undergo autolysis with much greater rapidity than the untreated diplococci.

Experiments were made to determine whether the diplococci. which were obviously altered without being killed, so that they underwent more rapid autolysis, would also show greater capacity for phagocytosis. The experiments were made as follows:

A 0.2 per cent. solution of sodium oleate was added to broth cultures to produce a concentration of 1 to 10,000 to 1 to 20,000 of the soap. After one hour's contact at room temperature the soap and broth were removed by washing the pneumococci twice in 0.85 per cent. salt solution in a centrifugal machine of high speed. The treated pneumococci were collected and made into a fixed suspension in salt solution, which suspension was used for the phagocytic tests. These tests were made with leucocytes alone and with leucocytes plus normal serum, and immune serum. Control observations were carried out with pneumococci of the same source which had been merely washed in salt solution. No phagocytosis had occurred in any of the preparations after the expiration of thirty minutes. On the other hand, while the number of diplococci remained unchanged in the control tubes and in the mixture of leucocytes and soaped diplococci alone, the tubes containing the soaped diplococci and the normal and immune sera showed a great reduction in the number of organisms; indeed, almost all had disappeared. Hence, it appears that the combined action of soap and serum led to a marked solvent or bacteriolytic effect upon the pneumococci, and at this early period in the observations no difference was noticeable between the action of the normal and the immune serum. But at the expiration of about eighteen hours a marked difference was apparent, for the tube containing the normal serum was now turbid, as a result of the multiplication of the small number of diplococci which had escaped destruction, while the tube containing the immune serum was sterile, since all of the diplococci had been dissolved. It could now be shown that in the solution of the soaped diplococci by means of serum, the leucocytes which had been added

for the purpose of ascertaining whether the treated cocci were phagocytable played no part, since the partial solution of the soaped organisms by the normal serum, and the complete solution by the immune serum, occurred likewise in the absence of the leucocytes.

THE EFFECTS OF SOAPED PNEUMOCOCCI UPON ANIMALS.

The preceding experiments were next modified to the extent that the various mixtures, instead of being allowed to remain in test tubes, were injected into the peritoneal cavity of rats and mice. In order to follow the series of changes taking place in the peritoneal cavity and to compare them with the changes occurring in test tubes, fluid was withdrawn by means of capillary tubes at various intervals after the injection.

In the case of the soaped pneumococci there appear within the first few hours an increasing number of leucocytes, and there takes place a rapid reduction in the number of organisms injected. In the case of the control series, in which pneumococci merely washed with salt solution have been injected, there is an absence of marked leucocytic exudation and an early and rapid multiplication of the diplococci. Phagocytosis is only occasionally met with in the exudate in either series of experiments. At the expiration of about eighteen hours all the control animals are found to have succumbed to a pneumococcus septicemia, while those which have been injected with the soaped diplococci alone and with the soaped organisms combined with normal serum are very ill or dying, and the peritoneal exudate obtained by means of capillary tubes contains many pus cells and innumerable diplococci. On the other hand, the mice that received the mixture of soaped pneumococci and immune serum are not noticeably ill. The peritoneal cavity, moreover, is found to contain a very small quantity of fluid in which there are no diplococci, very few pus cells, and relatively many mononuclear, probably desquamated endothelial cells, which often contain ingested polymorphonuclear leucocytes. All the mice of the experiment die within the day except those which received the immune serum; they recover. The capacity of the immune serum to render innocuous the soaped diplococci is very great, since, when for the rat the culture is of such virulence that 0.00001 cubic centi-

meter will kill a grown rat within two days, these animals withstand under the conditions mentioned the quantity of pneumococci contained in ten to fifteen cubic centimeters of the original culture. These results will be illustrated by a protocol which follows:

March 29, 1909. The plain broth culture, which was twenty-two hours old, was made from the heart's blood of a "passage" mouse. The soap solution was freshly prepared with Merck's sodium oleate. The serum consisted of a specimen of normal goat serum and of immune goat serum, the latter prepared by injecting highly virulent pneumococci intravenously into the goat. The soap solution was added to the broth culture in such quantity as to bring about a dilution of 1 to 20,000, and the mixture was allowed to rest for one hour at room temperature before being centrifugalized. The diplococci were rapidly washed twice with 0.85 per cent. salt solution in a centrifugal machine of high speed. The sedimented organisms were finally suspended in salt solution in such a manner as to make each 0.5 c.c. of the suspension represent 15 c.c. of the original culture. The control observations were made upon pneumococci treated in all respects in a similar manner, except that they had not been subjected to the action of the sodium oleate. Microscopical examination showed no appreciable difference in the soaped and the control diplococci. Equally profuse growths were obtained from each upon glucose serum agar. The quantity of the suspensions injected into the peritoneal cavity of each rat was 0.5 c.c.

Table I summarizes the results of the experiment.

TABLE I.

		Peritoneal fluid withdrawn.			Result.
		4 hours.	8 hours.	21 hours.	
Rat 41.	Control : washed diplococci alone.	No reduction in number of cocci ; few polymorphonuclear leucocytes.	Slight increase in number of cocci ; more p.n.l.		Died, 17 hrs.
Rat 37.	Washed diplococci + immune serum, 0.3 c.c.	No reduction in number of cocci ; few p.n.l.	Marked increase in number of cocci ; many p.n.l.		Died, 19 hrs.
Rat 36.	Soaped diplococci alone.	Few cocci ; very few p.n.l.	Few cocci ; very few p.n.l.	Abundant growth of cocci ; many p.n.l.	Died, 29 hrs.
Rat 44.	Soaped diplococci + normal serum, 0.3 c.c.	Marked reduction in number of cocci ; few p.n.l.	Marked reduction in number of cocci ; more p.n.l.	Abundant growth of cocci ; many p.n.l.	Died, 30 hrs.
Rat 47.	Soaped diplococci + immune serum, 0.3 c.c.	Only an occasional pair of cocci ; few p.n.l.	No cocci ; more p.n.l.	No cocci ; fewer p.n.l.	Recovered.

The animals that died presented at autopsy the usual findings from pneumo-coccic septicemia. Only an occasional example of phagocytosis in the peritoneal exudates.

This experiment, which is taken as an example from many of the same kind, shows clearly that the observations made *in vitro* on the action of soaps upon the pneumococci are applicable to the effects produced *intra vitam*. The conclusion, therefore, is justified that under the influence of minimal quantities of alkaline oleic acid soaps the pneumococci are so modified that they become more subject, not to phagocytosis, but to increased serum lysis, and that the effects of serum in this respect are much greater in the case of immune than in the case of normal serum. The effect, moreover, of the immune serum is to bring about the disappearance of the diplococci not by increasing phagocytosis, but by increasing lysis. Hence this experiment develops an important fact that was not ascertained in working with immune serum alone, for while the greater action of the immune serum over normal serum has, up to the present, been attributed to its greater bacteriotropic powers, it now appears that it possesses a greater lytic power as well.

THERAPEUTIC OBSERVATIONS.

Now that it has been shown that the combined effects of sodium oleate and an immune antipneumococcus serum suffice to protect rats which otherwise succumb to pneumococcus infection, when the immune serum is injected simultaneously with the soaped diplococci, the question arises whether any protection can be secured by in-jecting the immune serum at a later period and, hence, after infec-tion has already been accomplished. When the above experiment is repeated with the difference that the injection of immune serum is made after that of the soaped diplococci, it has been found that it is possible to prevent infection when the immune serum is injected within about one hour after the pneumococci, but not when it is injected later and after multiplication has already begun. Since the pneumococci employed have such a high degree of virulence, and the progeny of the soaped opsonins are not subject to serum lysis, this result was predictable.

It next became necessary to ascertain whether a favorable result

could be achieved when mixtures of soap and immune serum were injected into animals already infected with untreated virulent pneumococci. In planning experiments of this kind it was necessary first to take into account the already ascertained fact that bacteriolysis and hemolysis by means of soaps are inhibited in the presence of an excess of protein in the form of serum. Thus, if the culture of pneumococci, serum, and solution of soap be mixed together at once, the diplococci are not acted upon by the soap and hence are not prepared for solution, but multiply either in the test tube or in the body of the injected animal, in the latter instance causing death. If, therefore, any success in this line of experiment is to be achieved, a method must be found for keeping apart the soap and the serum at least until the soap has had time to unite with and act upon the pneumococci.

THE PREVENTION OF PROTEIN INHIBITION OF SODIUM OLEATE.

In considering the factors that act immediately in inhibiting the action of the soap upon the diplococci in the presence of serum, the first possibility thought of related to the calcium salts, which, by reacting with the sodium oleate might give rise to calcium oleate. As calcium oleate is insoluble, it would fail to act upon and alter the pneumococci. An effort was made, therefore, to remove the calcium of the serum by means of sodium oxalate; this oxalated serum was mixed with the culture. Sodium oleate was then added in quantity sufficient to kill the diplococci during one hour's contact in the absence of serum. This experiment, which was repeated, showed immediately that other factors besides the calcium entered into the reaction, since the diplococci were protected from the action of the soap by oxalated serum, just as they were by the native serum.

At this point advantage was taken of an observation made by von Liebermann and von Fenyvessy,[88] who noted that the inhibitory effect of serum upon soap hemolysis could be prevented by means of boric acid. The preliminary experiments with boric acid showed that in a 3 per cent. solution this chemical, when acting for the period of one hour, exhibited no appreciable antiseptic or

[88] *Ztschr. f. Immunitätsforsch.,* 1909, ii, 436.

destructive action on pneumococci. It could now be shown that when boric acid was added to the serum in a suitable quantity, it counteracted almost completely the inhibitory effects of the serum. The optimum quantity of the boric acid for the quantities of 0.2 cubic centimeter of serum and one milligram of sodium oleate proved to be from thirty to forty milligrams. When less than thirty milligrams was employed, the inhibition was imperfect; when more than forty milligrams was used, the boric acid itself deterred the action of the soap, and its effect on the serum inhibition became obscured.

Two-tenths cubic centimeter of serum, three milligrams of boric acid, and a quantity of sodium oleate yielding a dilution of 1 to 1,000 are allowed to act upon the pneumococci; it is found that all of those exposed have been killed, and nearly all dissolved. When less than thirty milligrams of boric acid are employed, a part only of the pneumococci are killed and dissolved, while if a greater amount be used, all of the cocci are killed, but few or none are dissolved.

EXPERIMENTS 10.14 AND 10.15. February 24, 25, 1910. Materials used: a twenty-four hour plain bouillon culture of "N. 1.21" made from the heart's blood of a "passage" mouse. 0.000001 c.c. fatal for a mouse. Freshly prepared 0.5 per cent. solution of sodium oleate. 5 per cent. solution of boric acid. Normal goat serum. The various mixtures are made in test tubes and allowed to remain for one hour. Then microscopical preparations and transplantations to glucose serum agar are made. Afterwards a part of each mixture is injected into the peritoneal cavity of a mouse so that each mouse receives 0.5 c.c. of culture, as shown in Table II.

Next, it was observed that the substitution of immune antipneumococcus serum for the normal serum favors the destruction of the diplococci and consequently the recovery of the inoculated animals. The difference in effects between the normal and the immune sera in these experiments is less striking than in the experiments of Table I, for the reason that here the concentration of soap used suffices in itself in the absence of serum to destroy the bacteria, and thus masks the effects of the immune serum. On the other hand, the greater efficiency of the immune over normal serum is shown in two distinct ways: first, by the smaller quantity of boric acid required to produce a given result with immune than with

TABLE II.

		Microscopical preparations.	Transplantations.	Result.
10.62	Culture + soap solution ; dilution, 1–1,000.	Outlines of cocci lost; small chromatin-staining masses remain.	Sterile.	Recovered.
10.72	Serum + culture + soap.	No reduction ; no morphological change.	Profuse growth.	Died, 17 hrs.
10.74	Boric acid (10 mg.) + serum + culture + soap.	Marked reduction in number ; all altered.	Profuse growth.	Died, 18 hrs.
10.75	Boric acid (20 mg.) + serum + culture + soap.	More reduction ; all altered.	Profuse growth.	Died, 30 hrs.
10.78	Boric acid (30 mg.) + serum + culture + soap.	Very few remain.	3 colonies.	Recovered.
10.79	Boric acid (40 mg.) + serum + culture + soap.	Only an occasional pair remains.	1 colony after 48 hrs.	Recovered.
10.80	Boric acid (60 mg.) + serum + culture + soap.	Slight reduction in number ; some swollen.	Sterile.	Died, 48 hrs.
10.76	Boric acid (60 mg.) + culture.	No change.	Profuse growth.	Died, 16 hrs.
10.73	Boric acid (20 mg.) + culture + soap.	Few pairs left; all altered.	Sterile.	Recovered.
10.77	Boric acid (60 mg.) + culture + soap.	Little or no reduction ; some swollen.	Sterile.	Died, 67 hrs.

All the animals that died, except Nos. 10.80 and 10.77, presented at autopsy the usual findings of death from pneumococcic septicemia. Nos. 10.80 and 10.77 died from the toxicity of the substances which were injected, and not from infection. In each there was a barely perceptible, thin peritoneal exudate, which was sterile, as was the heart's blood.

normal serum; and second, by the smaller quantity of immune than of normal serum required to protect the animals from infection. The former fact is brought out by the experiment summarized in Table III.

EXPERIMENT 10.16. February 28, 1910. The experiment is carried out in the same manner as experiments 10.14 and 10.15, except that immune goat serum is substituted for the normal serum.

TABLE III.

		Microscopical preparations.	Transplantations.	Result.
10.83	Boric acid (10 mg.) + immune serum + culture + soap.	Marked reduction in number of cocci ; all altered.	Profuse growth.	Recovered.
10.84	Boric acid (20 mg) + immune serum + culture + soap.	More reduction.	Scant growth.	Recovered.
10.85	Boric acid (30 mg.) + immune serum + culture + soap.	Only a few cocci remain.	1 colony.	Recovered.
10.86	Boric acid (40 mg.) + immune serum + culture + soap.	An occasional pair seen.	Sterile.	Recovered.
10.82	Control : boric acid (60 mg.) + immune serum + culture.	No change.	Profuse growth.	Died, 17 hrs.

10.82: autopsy showed usual findings of pneumococcic septicemia.

In this experiment the immunity principles of the serum have sufficed to compensate for the only partial removal of the serum inhibition which was afforded by the insufficient quantities of boric acid, for although viable diplococci were still present in mixtures 10.83 and 10.84, yet the injected animals recovered, while the corresponding ones, 10.74 and 10.75 of the preceding experiment with normal serum, succumbed.

The second way in which the superiority of the immune serum is exhibited is illustrated by the experiment summarized in Table IV, from which it will be seen that smaller quantities of immune serum accomplish what it is impossible for even larger quantities of normal serum to do. In order to keep the conditions of the experiment identical throughout and to secure an equal quantity of protein, normal serum was added to the immune serum to make the quantities of equal volume throughout, namely, 0.2 cubic centimeter, which is the amount required for complete inhibition of the action of the soap.

TABLE IV.

		Transplantations.	Result.
10.94	(Same as 10.74). Boric acid (10 mg.) + normal serum (0.2 c.c.) + culture ǀ soap.	Profuse growth.	Died, 40 hrs.
10.95	(Same as 10.75). Boric acid (20 mg.) + normal serum (0.2 c.c.) + culture + soap.	Moderate growth.	Died, 16 hrs.
10.96	Boric acid (10 mg.) + immune serum (0.05 c.c.) + normal serum (0.15 c.c.) + culture + soap.	Moderate growth.	Recovered.
10.97	Boric acid (20 mg.) + immune serum (0.05 c.c.) + normal serum (0.15 c.c.) + culture + soap.	Moderate growth.	Recovered.
10.98	Boric acid (10 mg.) + immune serum (0.02 c.c.) + normal serum (0.18 c.c.) + culture + soap.	Profuse growth.	Died, 40 hrs.
10.99	Boric acid (20 mg.) + immune serum (0.02 c.c.) + normal serum (0.18 c.c) + culture + soap.	Slight growth.	Died, 40 hrs.
10.100	Boric acid (10 mg.) + immune serum (0.01 c c.) + normal serum (0.19 c.c.) + culture + soap.	Profuse growth.	Died, 16 hrs.
10.101	Boric acid (20 mg.) + immune serum (0.01 c.c.) + normal serum (0.19 c.c.) + culture + soap.	Slight growth.	Died, 48 hrs.

From the preceding experiment it is apparent that as little as 0.05 cubic centimeter of the immune serum suffices to prevent infection and death even when as little as from ten to twenty milligrams of

boric acid are used in combination; while the larger quantity, namely, 0.2 cubic centimeter, of normal serum under the same conditions exerts little or no restraining action on the growth and infectivity of the pneumococci.

THERAPEUTIC TESTS WITH SERUM, SOAP, AND BORIC ACID MIXTURES.

Now that it has been ascertained that boric acid is capable of preventing the protein inhibition of soap *in vitro,* the question at once presents itself as to whether it will exercise a similar effect *intra vitam,* and if so, whether use can be made of soap and immune serum mixtures to prevent infections with the pneumococcus. With respect to this question it can be stated that rats which have received intraperitoneal injections of several times the fatal dose of a virulent culture of pneumococci may be saved from fatal infection by the subsequent injection of an appropriate mixture of immune serum, boric acid, and sodium oleate. In brief, it has been found that when what we shall call the therapeutic injection of the mixture described above is made to follow immediately the inoculation of the pneumococci or is made within one hour of the inoculation of the organisms, all of the animals so treated survive. When the treatment is postponed longer, so that the interval between the inoculation and the treatment is as long as two hours, about one-half of the treated animals survive, and when the treatment is still further postponed, the results are less satisfactory.

The experiment summarized in Table V is taken from a series, all the experiments of which resulted similarly.

EXPERIMENT 10.28. April 25, 1910. Materials used: a twenty-four hour plain broth culture of " N. 1.78 " made from the heart's blood of a "passage" mouse; 0.00001 c.c. fatal for a rat. A mixture, each cubic centimeter of which contains 0.2 c.c. of immune goat serum, 0.6 c.c. of a 5 per cent. solution of boric acid, and 0.2 c.c. of a 0.5 per cent. solution of sodium oleate.

The experiment was performed in duplicate, eight white rats being used in each series. The culture was injected first and then the therapeutic mixture, as shown in Table V.

The interpretation of the results of the therapeutic experiments appears to be as follows:

TABLE V.

10.153	10.161	Control : 0.01 c.c. culture.	Both died, about 40 hrs.
10.154	10.162	Control : 0.1 c.c. culture.	10.154 died, about 18 hrs.; 10.162 died, about 40 hrs.
10.155	10.163	0.01 c.c. culture + 0.5 c.c. thera-peutic mixture immediately.	Both recovered.
10.156	10.164	0.1 c.c. culture + 0.5 c.c. thera-peutic mixture immediately.	Both recovered.
10.157	10.165	0.01 c.c. culture + 0.5 c.c. thera-peutic mixture 2 hrs. later.	10.157 recovered ; 10.165 died, 3½ days.
10.158	10.166	0.1 c.c. culture + 0.5 c.c. thera-peutic mixture 2 hrs. later.	10.158 died, 4½ days ; 10.166 recovered.
10.159	10.167	0.01 c.c. culture + 0.5 c.c. thera-peutic mixture 2 hrs. and 24 hrs. later.	Both recovered.
10.160	10.168	0.1 c.c. culture + 0.5 c.c. thera-peutic mixture 2 hrs. and 24 hrs. later.	10.160 recovered ; 10.168 died, 5 days.

Although rat 10.165 had, at autopsy, a pneumococcic peritonitis and septicemia, its right lung was the seat of an extensive pseudo-tuberculosis, which is common in laboratory rats, and which may have contributed to its death.

In inhibiting the union of sodium oleate with the serum proteins, the action of boric acid is not perfect. It depends in part upon the amount of protein present and is greatest only under definite conditions. Hence, its effects *intra vitam* are greater during a period in which the protein of the serum exuded into the peritoneal cavity is not excessive, and its action becomes less perfect, and hence inadequate, when the exudate has become rich in serum protein. Moreover, the degree of multiplication of the pneumococci must also be taken into account since the action of the therapeutic mixtures upon the virulent diplococci is not indefinite; and in the experiments, multiples of the fatal dose having been inoculated, account must be taken of the multiplication occurring within the first two hours following the infection, which multiplication adds to the number of fatal doses present. Even the forming exudate in the peritoneal cavity is, by nature of the anatomical relations there present, likely to be enclosed by adhesions in pockets to which the therapeutic mixture may not have free access. Finally, the rat is, under the circumstances of the experiment, a species highly susceptible to infection, and particularly to septicemia; and within the period of two hours the pneumococci injected into the peritoneal cavity have gained access to the blood and through the blood to the internal organs, in which situations they are not readily, if at all, reached by the therapeutic mixture.

The problem presented in the rescue of these animals from a fatal infection is a severe one as compared with that presented by local affections due to the pneumococcus occurring in larger animals subject to spontaneous infection.

MECHANISM OF THE PROCESS.

The precise mechanism of the combined action of sodium oleate and immune serum, through which the growth of the pneumococci is so greatly restrained and their solution so much promoted, should be discussed. That the dilute solutions of the alkaline oleates produce definite and marked changes in the organisms that do not directly affect their viability is obvious. These changes are profound enough to bring the viable pneumococci within the dissolving sphere of serum, from which it would appear that one effect, at least, of the sodium oleate is to render the organisms more pervious for the serum. We wish to suggest that this action of the soap is connected in some degree with the lipoidal constituent of the organisms and possibly with the moiety contained within their outer membranes which, under the influence of the soap, having become attenuated or partly dissolved, permit ingress of the serum. To this conception of the manner of action of the soap would conform the greater tendency of the soaped pneumococci to undergo autolysis, since the weakening of the lipoidal barrier would favor the action, in the manner described by Hans Meyer,[39] of the autolytic ferments upon the bacterial cell. The bacterial cells thus altered by the action of the soap become apparently more pervious to an immune than to a normal serum, doubtless because of the presence in the former of principles, possibly of colloidal nature, which favor its entrance into the bacterial cell. Doubtless, also, the specific immunity principle exercises directly a destructive action upon the pneumococci, for the completion of which complement would seem not to be required.

Further experiment is required in order to ascertain to what extent the peculiar action of the alkaline soaps described is applicable to other members of the group of the pyogenic cocci. In this connection it should be recalled that the pneumococcus has already been

[39] *München. med. Wchnschr.*, 1909, lvi, 1577.

distinguished from other pyogenic cocci by reason of the fact that it is subject to solution by bile, while the others are not.   Studies are in progress for the purpose of determining the manner of reaction of other members of the pyogenic bacterial group to the alkaline soaps.

CONCLUSIONS.

Highly dilute solutions of the alkaline oleates, which do not suffice to alter appreciably the morphology or reproductive power of the pneumococci, nevertheless produce profound changes in their structure.

Pneumococci treated with sodium oleate become more subject to autolysis, as is indicated both by the rapidity and the perfection of the process of self-digestion, and at the same time they become subject to serum-lysis.

The serum-lysis of the soaped pneumococci tends to be incomplete with normal serum and to be perfect with an immune antipneumococcus serum.   When normal serum is employed, the surviving pneumococci subsequently multiply either in the test tube or in the animal body, in the latter case producing fatal infection. When, on the other hand, an immune serum is employed, lysis is complete, no multiplication occurs, the test tube mixture is sterilized, and the inoculated animal is protected from infection.

The inhibition of their activity which the soaps ordinarily suffer in the presence of protein, can be prevented by the addition of an appropriate quantity of boric acid, so that suitable mixtures of serum, soap, and boric acid can continue to exert a deleterious and solvent influence on the pneumococci, and the effect is greater when immune serum is employed in the mixtures.

Infection can not only be prevented when the mixture of immune serum, soap, and boric acid is added to the pneumococci before injection into the peritoneal cavity of small animals, but the infection can likewise be prevented when a therapeutic injection of a mixture of the three substances mentioned is made to follow the inoculation of normal, highly virulent diplococci.   The limits of the activity of the therapeutic mixture are determined, in part by the amount of protein to be overcome, and in part by the peculiarities of the infection occurring in highly susceptible animal species.

The virulence of the pneumococci is somewhat diminished by the soap treatment, but the treated organisms are not rendered more subject to phagocytosis.

It would appear that the action of the soap is exerted upon the lipoidal moiety of the bacterial cells, through which they are rendered more pervious to serum constituents and brought under their deleterious and dissolving influence. The changes in the pneumococci here described probably have a prototype in the resolving exudate of a pneumonic process, so that it may be considered that they occur in the animal body in the course of spontaneous infection and constitute one of the conditions of the conquest of the organism by the body's forces. A certain conformity exists between the manner of destruction of the pneumococci in a pneumonic exudate and that in the artificially prepared soap-serum mixtures.

In order to imitate outside of the body the conditions of the removal of pneumococci within the body, it does not suffice merely to study the reactions to leucocytes and serum and to conclude from these reactions the means which the body employs for the disposal of the diplococci; but it is necessary to invoke still other factors, among which are the effects of chemical substances present in exudates, of which the soaps represent one class. The failure hitherto to unify the reactions in test tubes with those occurring in the body in connection with the pneumococcus may be due to the fact that account was not taken of this class of chemical bodies.

Whether the principle here presented can be made applicable to the treatment of local pneumococcus infections in human beings is a pressing question. Its application to the treatment of local infections, to the seats of which the serum, soap, and boric acid mixtures can be directly applied, particularly after evacuation of an inflammatory exudate, seems to offer promise.

BIOCHEMICAL STUDIES ON SO–CALLED SYPHILIS ANTIGEN.[1]

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INTRODUCTION.

The mechanism of the Wassermann reaction is still unsolved. We know merely this: that complement in the presence of syphilitic antigen may be rendered inactive by one or more substances in the body fluids of a syphilitic or parasyphilitic patient. Our present paper deals with the nature of these antigenic substances.

Wassermann, Neisser, and Bruck discovered that the aqueous extract of the liver of a congenitally syphilitic fetus possessed antigenic properties. Ladislaus Detre found independently, and almost si-

[1] Received for publication, September 21, 1910.

multaneously, a similar property in the extracts prepared from condyloma and other syphilitic tissues. Subsequent investigations of Marie and Levaditi brought out the fact that an aqueous extract of a non-syphilitic liver has a weaker but similar action.

A remarkable revelation soon followed through the studies of Landsteiner, Müller, and Pötzl, and later through those of Meier and Porges, who discovered that the antigenic substances can be extracted by means of alcohol from the human or animal liver or heart. This finding has opened up an entirely new phase of the doctrine of the well known phenomenon of complement fixation, and separates the Wassermann reaction from the classical examples of the Bordet-Gengou reaction due to specific antigens and antibodies.

Additional studies by Levaditi and Yamanouchi, Meier and Bauer, Fleischmann, Sachs and Rondoni, Noguchi, Browning and McKenzie, Leathes and Fitzgerald, and many others, confirmed and extended the original observations of Landsteiner, Müller, and Pötzl, and of Porges and Meier. As the result of these researches, the following facts may be considered as established: the Wassermann reaction can be produced with lecithin, certain phosphatids of liver, heart, or kidney, and, in a lesser degree, with certain bile salts, all of which substances are extractable with alcohol. It is also contended that the entire alcoholic extract of organs, lecithins, and some phosphatids, do not give as high a percentage of positive reactions in syphilis or metasyphilis as a suitable aqueous extract of syphilitic fetal liver. This seems to indicate that the aqueous extract contains something in addition to the lipoids and salts which makes the reaction stronger and more specific. This assumption, however, has not been proved.

It is well known that some specimens of congenitally syphilitic fetal livers do not manifest antigenic properties. One is compelled, therefore, to make a thorough trial before concluding that an extract may be used as antigen.

In the case of aqueous extracts, the selection of a suitable one requires much care. The same care has not been exercised in selecting alcoholic extracts and lipoids. In the case of the lipoids, especially lecithin, this lack of precaution is due largely to the use of a

definite chemical term. When speaking of lecithin, one is accustomed to think of it as a chemical and to overlook its biological properties. The same is true of phosphatids. This chemical interpretation of the terms has led many investigators to rely on any preparation of lecithin or phosphatids of tissues without biological discrimination. A worker often uses lecithin because it has been recommended by some investigators. Noguchi, who has been criticised for recommending lecithin, has always coupled the term with "selected." For this and other reasons he no longer employs the term lecithin in designating the aceton-insoluble fraction of tissue lipoids.

In testing lipoids derived from beef kidney and fractionated in twenty-one different ways by Professor Edward K. Dunham, one of us (Noguchi) found that only nine possessed antigenic properties. Of the nine, three were soluble in aceton and six (phosphatids) were insoluble. Only one of the latter was composed of lecithin. Noguchi found, moreover, on comparing the aceton-insoluble fraction of the blood clot and serum of syphilitic and normal persons with the corresponding fractions of syphilitic and normal livers, that the antigenic activity of the former was not inferior to that of the latter. Moreover, the aceton-soluble fraction of the liver and especially that of the blood coagulum and serum possessed antigenic properties. After studying its hemolytic and anti-complementary properties, Noguchi advised the exclusive use of the aceton-insoluble fraction instead of the entire unfractionated alcoholic extract, for the reason that the aceton-insoluble lipoids possess the highest antigenic action and are unaccompanied by other undesirable properties. By eliminating the other constituents (such as neutral fats, fatty acids, soaps, and certain proteid materials) contained originally in the alcoholic extract, Noguchi succeeded in avoiding the non-specific complement fixation which occasionally occurred in unheated human serum. Here arose the important question: What is the criterion by which one can judge the antigenic fitness of the aceton-insoluble fraction? Noguchi had met with a number of preparations which were unsuitable either on account of the total absence or weakness of the antigenic properties or on account of the presence in the emulsion of some hemolytic or anti-

complementary substance.   These irregularities made it necessary to study analytically a large number of tissues with special reference to the composition of the alcoholic extract—the antigenic, hemolytic, and anticomplementary properties of different fractions, and the chemical constitution of the more important ones.

These were the problems which confronted us and they have led to the work which forms the basis of the present article.   We believed that it was a matter of great importance to approach the question experimentally, for reducing the problem to a biochemical basis removes from the Wassermann reaction its chief difficulty.

METHOD OF STUDY.

EXTRACTION AND FRACTIONATION.

The organ was cut into very small pieces, mixed with four volumes of 95 per cent. alcohol and preserved in a sealed jar. With occasional shakings, it was left at room temperature for six to seven weeks. The alcohol was then passed through filter paper and a second extract was made by treating the residue with four volumes of absolute alcohol at 37° C. for four days, with daily shaking. The second extract was collected by filtration, united with the first and the whole was placed in a very wide crystallization dish and evaporated by means of a current of air at 37° C. After desiccation the sticky mass was weighed and then treated repeatedly with ether to separate the ether-soluble substances. The ether-insoluble part was weighed and the ether-soluble portion was evaporated and weighed and then dissolved in a small quantity of ether and mixed with ten volumes of water-free aceton. The large amount of precipitate formed was usually separated by careful decantation—sometimes, however, filtration through hardened filter paper was necessary. The aceton-insoluble precipitate (phosphatids) was collected, freed from aceton by evaporation, and weighed. The aceton-soluble fraction (neutral fats, fatty acids, cholesterin, etc.) was freed from aceton by evaporation and weighed.

The various fractions derived from the tissue may be represented schematically as follows:

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                    Tissue
                      |
            Alcoholic extract
                      |
          ┌───────────┴───────────┐
Ether-insoluble fraction     Ether-soluble fraction
                                  |
                      ┌───────────┴───────────┐
           Aceton-insoluble fraction     Aceton-soluble fraction.
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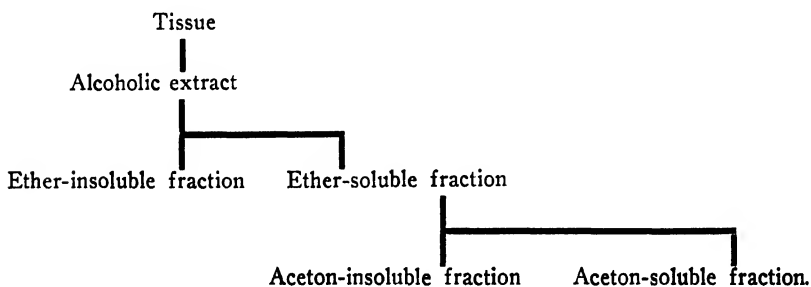
The method indicated above was considered sufficiently accurate for our purpose, as we intended to resort to a more thorough analysis in our next undertaking.

The solubility in hot alcohol of the ether-insoluble fraction was ascertained for a few specimens. The fractions soluble and insoluble in hot alcohol proved to be soluble in water. The insoluble fraction is composed of certain protein substances which, together with various salts, are held in apparent solution in the alcoholic extract. The hot alcohol-soluble fraction is composed chiefly of salts of saturated and unsaturated fatty acids and of bile acid salts.

The substances contained in the fractions soluble and insoluble in hot alcohol are important sources of error in complement fixation tests.

IODINE VALUE AND ACIDITY.

The iodine value of the aceton-insoluble fraction of each undehydrated specimen was determined by the method of Hübl. For this purpose the light brownish mass, which had been under a layer of water-free aceton in a sealed tube, was taken out of aceton and air-dried until the aceton evaporated. This required about fifteen minutes.

The acidity of the aceton-soluble fraction was determined after the aceton had been completely evaporated. The oily substance was weighed out and 0.025 gm. was dissolved in 10 c.c. of ethyl alcohol and the amount of $N/100$ potassium hydroxide necessary to neutralize 1 c.c. was determined, the indicator, phenolphthalein, remaining red for one minute.

BIOLOGICAL PROPERTIES.

Systematic and parallel examinations were made to determine the inherent hemolytic, the anticomplementary, and the antigenic properties of the fractionated fats and lipoids.

Preparation of Emulsions.—The emulsions were uniformly 0.25 per cent., that is, 0.025 gm. of material was suspended in 10 c.c. of a 0.9 per cent. sodium chloride solution. In the case of the aceton-soluble fraction, however, accurate work was impossible, for, in isotonic salt solution (0.9 per cent. sodium chloride), it proved exceedingly difficult to make a stable emulsion of this fraction. The aceton-insoluble fraction, however, easily emulsified by mixing salt solution with a concentrated solution of the material in ether or alcohol. Even when the material was not completely soluble in ether or alcohol, a good emulsion was obtained by adding salt solution. The emulsions of the latter were clear and opalescent. They were usually neutral to litmus and remained stable during many months.

Ether-insoluble Fraction.—The quantity of ether insoluble material obtained from different livers varied greatly. Autolyzed organs yielded the largest amount.

The biological factors in the ether insoluble fraction were not studied systematically in the experiments here reported. One of us (Noguchi), however, had already made a special examination of it and found that the hot alcohol-*insoluble* material of this fraction sometimes caused, with a certain human serum, a non-specific fixation of complement, and that the hot alcohol-*soluble* fraction

always exhibited a powerful hemolytic property. The amount of the hot alcohol-soluble fraction in the ether-insoluble material varied greatly, being sometimes very small, sometimes large.

Determination of the Biological Properties.—(*a*) To determine the *inherent hemolytic property,* gradually increasing fractions of 1 c.c. of the emulsions were put into a series of test tubes and 0.9 per cent. sodium chloride solution was added to bring the contents of each tube up to 1 c.c. Into each tube was then introduced 0.1 c.c. of a 10 per cent. suspension of washed human corpuscles. The tubes were shaken and incubated at 37° C. for two hours. The quantities of emulsion in the table are for complete hemolysis.

(*b*) To determine the *anticomplementary property,* increasing fractions of 1 c.c. of the emulsions, as above, were placed in a series of tubes, and then into each, one unit of complement (fresh guinea pig serum), usually 0.1 c.c. of a 20 per cent. dilution, was introduced and enough salt solution was added to make the contents of each tube equal to 1 c.c. The tubes were incubated at 37° C. for one hour in an air thermostat, or for thirty minutes in a constant temperature water bath. After incubation, to each tube 0.1 c.c. of a 10 per cent. suspension of washed human corpuscles and one unit of antihuman amboceptor (rabbit) were added. The mixture, with frequent shakings, was now incubated for two hours, and the result was recorded. The quantities of emulsion given in the table are for complete inhibition.

(*c*) To determine the *antigenic property,* a mixture of strongly positive syphilitic sera from six or more persons was employed. These sera were usually active, but sometimes they were inactivated (*i. e.,* when anticomplementary). It was necessary, first, to determine the smallest amount of serum capable of causing complete fixation, that is, the unit of syphilitic antibody. In order to do this, it was necessary to have a standard antigen emulsion. This we had, and its strength and quality had been proved in connection with an innumerable number of positive as well as non-syphilitic sera. In this way we determined the smallest quantity of syphilitic serum (antibody) that could be detected by using a good antigen, and this quantity of the serum was used for testing the strength of our different preparations of emulsion. In our tests gradually increasing quantities of the emulsion were measured into a series of test tubes, to which were also added one unit of syphilitic antibody and two units of complement, that is, 0.1 c.c. of a 40 per cent. dilution of fresh guinea pig serum. As usual, the contents of each tube were made equal to 1 c.c. by adding salt solution. The mixture was incubated at 37° C. for one hour, and then 0.1 c.c. of a 10 per cent. suspension of washed human corpuscles and two units of antihuman amboceptor were added to each tube. The second incubation was for two hours. The results were taken a few hours after stopping the incubation.

The quantities of emulsion recorded in the accompanying table are those which caused complete fixation. The largest amount of emulsion employed was 0.4 c.c., and any emulsion which, in this quantity, failed to cause fixation was regarded as possessing no antigenic property.

MATERIAL FOR INVESTIGATIONS.

Animal and human livers and animal hearts and brains were studied. The total number of all tissues investigated was eighty-seven. Animal livers were

obtained fresh in the laboratories of the Rockefeller Institute and placed at once in alcohol (95 per cent.). To our collection Dr. Dochez, of the Institute, kindly added the alcoholic extract of an autolyzed dog liver.

Livers were secured from the following animals: rabbits, 4; guinea pigs, 5; and dogs, 4. In addition to these, brains were obtained from eight guinea pigs and two dogs, and hearts from eight guinea pigs. On account of their smallness the brains and hearts of the guinea pigs were extracted together.

Human livers were secured through the courtesy of Dr. James W. Moore, Central Islip State Hospital, New York; Dr. Charles Rusk, Manhattan State Hospital, New York; Dr. John A. Wiseman, Kings Park State Hospital, New York; Dr. Martha Wollstein, Babies' Hospital, New York City; Dr. Mortimer Warren, Roosevelt Hospital, New York City; and Dr. Benjamin Schwartz, Bellevue Hospital, New York City. For their kind coöperation we wish to express our thanks.

The human livers were obtained from patients who had suffered with the following: dementia paralytica, 16; dementia precox, 8; dementia senilis, 2; manic depressive insanity, 1; senile psychosis, 10; epileptic psychosis, 1; cerebral hemorrhage, 1; pulmonary tuberculosis, 4; tubercular meningitis, 2; tubercular peritonitis, 1; erysipelas, 2; acute peritonitis, 4; lobar pneumonia, 3; bronchopneumonia, 1; acute enteritis, 1; carcinoma ventriculi, 1; cirrhosis of liver, 1; pulmonary thrombosis, 1; insufficiency of mitral and aortic valves, 1; congenital heart malformation, 1; severe anemia, 1; cerebral abscess, 1; uremia, 1; and chronic alcoholism, 1. Livers were also secured from an undiagnosed psychiatric patient, from an imbecile, from a nine months fetus, and from a premature child. The total number of human livers employed in these experiments was seventy.

With one exception all autopsies were performed within twenty-four hours after death. In the exceptional case eighty-two hours had elapsed. The livers were collected at autopsy and put immediately into four volumes of 95 per cent. alcohol.

Our complete experimental protocols are given in one general table and the results are described in the following pages. The results are further analyzed according to certain special considerations, such as the frequency and distribution of various biological properties of the fractionated tissue extracts, with reference to clinical diagnosis, pathological conditions of the tissues, and the age of the patients.

RESULTS OF EXPERIMENTS.

I. GENERAL PRESENTATION AND DISCUSSION OF RESULTS.

In Table I we have brought together all experimental data in such form that one can easily see the quantities of the different fractions of our tissues and their biological properties. It also gives the iodine number and acidity of some of the fractions of each specimen.

TABLE I.

#		Age	Weight of tissue in gm.	Fractionation					Biological properties of the ether-soluble tissue constituents, together with determination of the iodine values and acidity.							
									Aceton-insoluble fraction.				Aceton-soluble fraction.			
				Weight of substances extracted with alcohol in gm.	Weight of substances insoluble in ether in gm.	Weight of substances soluble in ether in gm.	Weight of substances insoluble in aceton in gm.	Weight of substances soluble in aceton in gm.	0.25 per cent. emulsion.			Iodine value.	0.25 per cent. emulsion.			Acidity N/10 potassium hydroxide.
									Antigenic (in c.c.).	Hemolytic (in c.c.).	Anticomplementary (in c.c.).		Antigenic (in c.c.).	Hemolytic (in c.c.).	Anticomplementary (in c.c.).	
1	Liver (normal): dementia paralytica, carbon monoxide poisoning 2 months before death.	62 yr.	25	2.4	0.5	1.9	0.77	1.1	0.02			59.00	0.1	0.03		0.5
2	Liver: dementia paralytica (tabetic), pneumonia, nephritis, syphilis.	47 yr.	24	2.7	0.2	1.5	0.92	0.56	0.02			54.61	0.1	0.15		0.25
3	Liver (passive congestion): dementia paralytica.	35 yr.	24	2.15	0.4	1.69	0.85	0.32	0.04			46.99	0.05	0.15		0.175
4	Liver (normal): dementia paralytica, broncho-pneumonia, nephritis.	62 yr.	23	2.	0.35	1.65	0.85	0.75	0.04			51.43	0.1	0.15		0.25
5	Liver (anemic): dementia paralytica, pachymeningitis hemorrhagica, pneumonia.	35 yr.	25	1.69	0.35	1.3	0.62	0.62	0.05			38.78	0.05			0.2
6	Liver (passive congestion): dementia paralytica, pneumonia, pulmonary emphysema, syphilis.	40 yr.	23	1.95	0.52	1.29	1.02	0.25	0.03		0.4	44.45	0.05	0.15	0.1	0.25
7	Liver (passive congestion): dementia paralytica (tabetic), acute mitral valvulitis, miliary abscesses of kidneys, cystitis.	36 yr.	26	2.95	1.6	1.4	0.95	0.35	0.04			46.85		0.2	0.1	0.275
8	Liver (passive congestion): dementia paralytica, chronic aortitis, double pyelonephritis.	48 yr.	28	2.5	0.75	1.75	0.95	0.8	0.04		0.2	49.47		0.15	0.15	0.35

No.		Age												
9	Liver (normal): dementia paralytica, acute nicotin poisoning	50 yr.	1.95	0.45	1.5	0.9	0.6	0.05	0.2	49.47		0.15	0.2	0.175
10	Liver (passive congestion): dementia paralytica, chronic nephritis	57 yr.	2.35	0.95	1.4	0.8	0.5	0.04	0.2	44.45	0.15		0.2	0.225
11	Liver (passive congestion): dementia paralytica, pulmonary tuberculosis, nephritis	51 yr.	2.05	1.15	0.85	0.45	0.4	0.04	0.15	46.35	0.1	0.2	0.2	0.225
12	Liver (passive congestion): dementia paralytica, aortitis syphilitica, broncho-pneumonia	55 yr.	1.9	0.69	1.2	0.82	0.36	0.05		43.81	0.1	0.15	0.2	0.25
13	Liver (normal): dementia paralytica, pneumonia, nephritis	58 yr.	1.69	0.35	1.31	0.74	0.47	0.05	0.2	38.10	0.1	0.15	0.2	0.225
14	Liver (fatty): dementia paralytica, chronic nephritis	50 yr.	2.45	0.91	1.53	0.92	0.5	0.05 Slight fixation		31.75		0.1	0.2	0.225
15	Liver (passive congestion): dementia paralytica, chronic nephritis, arteriosclerosis	60 yr.	2.1	0.75	1.34	0.6	0.64	0.03		50.80	0.1	0.15	0.2	0.25
16	Liver (fatty): dementia paralytica, aortitis syphilitica, pulmonary edema	49 yr.	2.	0.68	1.3	0.61	0.58	Slight fixation		31.11	0.1	0.1	0.2	0.3
17	Liver (normal): dementia senilis, chronic nephritis, ascites, hydrothorax	65 yr.	3.2	0.3	2.85	1.35	1.47	0.04		43.81	0.1	0.1		0.15
18	Liver (normal): dementia senilis (?), brown atrophy of heart, thrombosis of aorta, arteriosclerosis	60 yr.	3.33	1.2	2.1	1.2	0	0.05	0.2	40.64	0.1			0.125
19	Liver (sclerosis and perihepatitis): senile psychosis, pulmonary tuberculosis, nephritis, amyloid degeneration of spleen	59 yr.	3.	1.	2.	0.6	1.4	0.02	0.2	47.62	0.2		0.15	0.2
20	Liver (normal): senile psychosis, generalized arteriosclerosis, broncho-pneumonia, nephritis, gangrene of foot	63 yr.	2.3	0.43	1.8	0.97	0.8	0.05		38.73		0.25		0.225
21	Liver (normal): senile psychosis, pulmonary tuberculosis, chronic nephritis	63 yr.	2.	0.4	1.55	0.83	0.72	0.03	0.4	47.62	0.2		0.2	0.25

TABLE 1 (CONTINUED).

			Fractionation.						Biological properties of the ether-soluble tissue constituents, together with determination of the iodine values and acidity.							
									Aceton-insoluble fraction. 0.25 per cent. emulsion			Iodine value.	Aceton-soluble fraction. 0.25 per cent. emulsion			Acidity N/10 potassium hydroxide.
		Age.	Weight of tissue in gm.	Weight of substances extracted with alcohol in gm.	Weight of substances insoluble in ether in gm.	Weight of substances soluble in ether in gm.	Weight of substances insoluble in aceton in gm.	Weight of substances soluble in aceton in gm.	Antigenic (in c.c.).	Hemolytic (in c.c.).	Anticomplementary (in c.c.).		Antigenic (in c.c.).	Hemolytic (in c.c.).	Anticomplementary (in c.c.).	
22	Liver (normal): senile psychosis, hypertrophy of heart.	63 yr.	25	2.73	1.15	1.55	1.	0.55	0.04			48.89	0.05	0.2	0.1	0.225
23	Liver (passive congestion): senile psychosis, pneumonia hypostatica.	64 yr.	24	2.6	0.75	1.87	0.8	1.05	0.05			43.81		0.1		0.25
24	Liver (normal): senile psychosis, moderate cerebral atrophy, general arteriosclerosis, chronic nephritis.	88 yr.	25	2.15	0.85	1.25	0.75	0.5	0.05			44.45		0.2		0.2
25	Liver (normal): senile psychosis, chronic nephritis, choleocystitis.	86 yr.	25	2.48	0.4	2.02	1.05	0.95	0.03		0.2	49.47	0.3			0.175
26	Liver (82 hours post mortem) senile psychosis, chronic nephritis, cholelithiasis.	67 yr.	29	1.95	1.05	0.95	0.42	0.52	0.05			42.54		0.25		0.2
27	Liver (fatty): senile psychosis, dilatation of heart, infarct of kidney.	65 yr.	30	1.85	0.57	1.27	0.69	0.58	No fixation			21.94				
28	Liver (passive congestion): senile psychosis.	60 yr.	30	2.25	0.32	1.9	0.82	1.05	0.04			44.45	0.1	0.1	0.2	0.3
29	Liver (normal): dementia precox, carcinoma of pancreas.	55 yr.	24	3.	1.13	1.82	0.57	1.25	0.04			46.35	0.05	0.1		0.25
30	Liver (normal): dementia precox, miliary tuberculosis of lungs and spleen.	44 yr.	24	2.95	0.8	2.07	0.9	1.1	0.03			50.80		0.05		0.225

No.	Organ (condition): diagnosis	Age														
31	Liver (passive congestion): dementia precox, chronic nephritis....	62 yr.	25	2.3	0.8	1.45	0.9	0.5	0.04		0.4	50.03		0.07	0.05	0.175
32	Liver (passive congestion): dementia precox, broncho-pneumonia	50 yr.	21	1.65	0.4	1.3	0.65	0.65	0.04			39.37		0.05	0.05	0.225
33	Liver (normal): dementia precox, pulmonary tuberculosis......	50 yr.	25	2.5	1.15	1.35	0.9	0.45	0.02			56.57		0.05		0.35
34	Liver (normal): dementia precox..	27 yr.	30	3.	1.3	1.65	0.95	0.7	0.04			48.26	0.15		0.2	0.175
35	Liver (normal): dementia precox, pulmonary tuberculosis......	31 yr.	27	2.95	1.2	1.75	0.95	0.8	0.02			59.00		0.1		0.225
36	Liver (normal): dementia precox, pulmonary tuberculosis......	37 yr.	13	0.77	0.15	0.6	0.27	0.31	0.05			38.19		0.15		0.2
37	Liver (very soft and friable): epileptic psychosis; died in status; broncho-pneumonia..	52 yr.	30	2.6	0.9	1.65	1.2	0.45	0.02			52.07	0.1	0.05		0.25
38	Liver (normal): manic depressive insanity, septicemia, abscesses of kidney....	47 yr.	35	1.47	0.3	1.15	0.85	0.3	0.04		0.2	43.81		0.1		0.25
39	Liver (fatty); imbecile, peritonitis septica..	45 yr.	30	2.3	0.22	2.2	0.84	1.29	0.07			34.35	0.05	0.05	0.2	0.25
40	Liver (marked passive congestion): cerebral hemorrhage, pulmonary tuberculosis....	40 yr.	32	3.2	0.97	2.15	0.95	1.19	0.05		0.4	37.46	1.15		0.3	0.2
41	Liver (normal): pulmonary tuberculosis....	3 yr.	30	2.3	1.1	1.15	0.4	0.75		0.3				0.01	0.05	0.25
42	Liver (normal): pulmonary tuberculosis....	3 yr.	30	2.05	0.7	1.3	0.8	0.5	0.03		0.2	49.47	0.05	0.1	0.1	0.15
43	Liver (normal): tubercular meningitis..	4 yr.	30	3.2	1.2	2.	1.2	0.75	0.03		0.2	56.54	0.075	0.1		0.25
44	Liver (normal): pulmonary tuberculosis....	35 yr.	35	4.2	2.5	1.7	0.6	1.05	0.05			34.47	0.025	0.025		0.45
45	Liver (normal): pulmonary tuberculosis....	18 yr.	30	2.05	0.75	1.3	0.7	0.6	0.04			41.91		0.2	0.1	0.15
46	Liver (normal): tubercular peritonitis..	30 yr.	30	3.	1.35	1.65	0.85	0.8	0.04		0.2	48.73	0.1	0.025	0.2	0.375
47	Liver (normal): tubercular meningitis..	8 mo.	34	1.6	0.4	1.2	0.85	0.32	0.02		0.2	53.34		0.05	0.05	0.2
48	Liver (normal): erysipelas......	7 mo.	32	1.87	1.1	0.75	0.6	0.15	0.05		0.2	42.54		0.025	0.025	0.3
49	Liver (normal): erysipelas......	6 wk.	32	2.95	0.6	2.2	0.75	1.4	0.02		0.2	50.80		0.05	0.05	0.2

TABLE I (Continued).

| | | | Fractionation. | | | | | | Biological properties of the ether-soluble tissue constituents together with determination of the iodine values and acidity. | | | | | | | |
| | | | | | | | | | Aceton-insoluble fraction. | | | | Aceton-soluble fraction. | | | |
		Age.	Weight of tissue in gm.	Weight of substances extracted with alcohol in gm.	Weight of substances insoluble in ether in gm.	Weight of substances soluble in ether in gm.	Weight of substances insoluble in aceton in gm.	Weight of substances soluble in aceton in gm.	Antigenic 0.25 per cent. emulsion (in c.c.).	Hemolytic (in c.c.).	Anticomplementary (in c.c.).	Iodine value.	Antigenic 0.25 per cent. emulsion (in c.c.).	Hemolytic (in c.c.).	Anticomplementary (in c.c.).	Acidity N/10 potassium hydroxide.
50	Liver (normal): acute peritonitis..	3 mo.	25	1.9	0.37	1.5	0.95	0.5	0.02			52.04	0.05	0.1		0.2
51	Liver (normal): acute peritonitis, gastric ulcer	19 yr.	25	1.8	0.8	1.	0.8	0.2	0.04		0.1	40.00		0.025		0.425
52	Liver (normal): acute peritonitis, typhoid fever.........	25 yr.	32	2.65	1.05	1.55	0.9	0.65	0.03			45.72	0.05	0.025		0.275
53	Liver (normal): acute peritonitis..	25 yr.	30	2.8	0.8	1.9	0.95	0.92	0.02			50.80		0.075		0.275
54	Liver (normal): pneumonia and influenza..............	7 mo.	30	2.54	0.7	1.8	1.25	0.45	0.04			39.37	0.05	0.1		0.25
55	Liver (normal): pneumonia......	34 yr.	24	2.98	1.	1.95	0.95	1.	0.03		0.2	44.45	0.2	0.25		0.4
56	Liver (congested): pneumonia....	50 yr.	32	2.27	0.85	1.47	0.8	0.65	0.04			41.27		0.1		0.275
57	Liver (normal): pneumonia......	7 mo.	25	2.55	0.8	1.75	0.75	1.05	0.05			40.00	0.075	0.1		0.2
58	Liver (normal): acute enteritis...	3 wk.	28	2.1	0.57	1.73	1.2	0.5	0.02			53.37		0.1		0.375
59	Liver (normal): cancer of stomach	40 yr.	30	3.35	1.53	1.8	1.28	0.5	0.02		0.2	54.61		0.1		0.25
60	Liver (normal): cirrhosis of liver..	55 yr.	35	2.95	0.97	2.05	1.1	0.9	0.02		0.2	50.80		0.025		0.225
61	Liver (normal): pulmonary thrombosis..................	50 yr.	25	1.85	0.65	1.25	0.65	0.58	0.05			39.37	0.2			0.2
62	Liver (passive congestion): cardiac insufficiency............	60 yr.	30	3.73	2.3	1.45	0.75	0.7	0.05			38.10		0.025		0.375
63	Liver (normal): malformation of heart; serum reaction positive..	1 wk.	30	2.3	1.05	1.25	0.95	0.3	0.03			50.80	0.15			0.225
64	Liver (normal): grave anemia....	30 yr.	30	4.85	3.55	1.3	0.3	1.	0.02		0.2	53.34		0.025		0.5
65	Liver (normal): cerebral abscess..	36 yr.	30	3.2	2.78	2.35	1.28	1.07	0.02		0.1	60.27	0.1	0.15		0.2
66	Liver (normal): uremia.........	50 yr.	25	2.85	1.05	1.8	1.15	0.65	0.04			41.27		0.025		0.475
67	Liver (normal): alcoholism.......	53 yr.	24	3.8	1.3	2.5	0.6	1.85	0.04		0.2	58.36		0.15	0.1	0.25

No.		Age													
68	Liver (normal): premature child, atelectasis		30	3.1	1.1	2.1	0.9	1.1	0.02		54.61	0.05	0.15		0.275
69	Liver (normal): 9 month fetus; mother, negative serum reaction		30	2.8	0.8	1.9	0.65	1.17	0.02		55.88		0.1		0.25
70	Liver (normal): died in excitement; hemorrhagic infarct of the lungs	35 yr.	30	2.55	0.42	2.08	1.1	0.9	0.02		64.73		0.025		0.2
71	Liver (rabbit)	Full grown	26	2.1	0.7	1.45	0.95	0.5	0.04	0.2	44.45	0.15	0.15	0.2	0.225
72	Liver (rabbit)	"	32	2.75	0.9	1.85	0.75	1.1	0.04	0.15	41.27	0.15	0.2	0.2	0.25
73	Liver (rabbit)	"	30	2.8	0.6	2.2	1.5	0.7	0.03	0.15	47.63		0.15	0.1	0.225
74	Liver (rabbit)	"	34	3.05	1.15	1.9	1.35	0.52	0.05	0.2	34.47	0.15		0.2	0.2
75	Liver (guinea pig)	"	35	2.3	0.7	1.58	0.87	0.7	0.04		44.45	0.15		0.2	0.2
76	Liver (guinea pig)	"	30	2.2	0.5	1.7	1.2	0.5	0.04	0.1	41.90			0.25	0.225
77	Liver (guinea pig)	"	30	1.2	0.5	0.7	0.5	0.2	0.04	0.2	39.37		0.1		0.3
78	Liver (guinea pig)	"	25	2.38	0.9	1.4	1.1	0.3	0.04		42.91			0.2	0.2
79	Liver (guinea pig)	"	32	2.1	0.7	1.45	1.05	0.4	0.04	0.15	43.18	0.15	0.2	0.2	0.175
80	Liver (dog)	"	25	1.85	0.7	1.15	0.7	0.45	0.03	0.2	46.35	0.15	0.15		0.225
81	Liver (dog)	"	30	2.42	1.1	1.32	0.92	0.45	0.05		38.10	0.05		0.2	0.225
82	Liver (dog)	"	30	2.1	0.8	1.3	0.7	0.6	0.03	0.2	46.35		0.1		0.25
83	Liver (dog): autolyzed	"	30	7.	4.85	2.1	1.25	0.8	0.02		69.85	0.15	0.03	0.15	0.5
84	Heart (from 8 guinea pigs)	"	8	0.98	0.23	0.75	0.53	0.22	0.03	0.2	49.47	0.15	0.15	0.2	0.175
85	Brain (from 8 guinea pigs)	"	20	2.52	0.55	1.95	1.3	0.65	0.1	0.2	57.78	0.1	0.15	0.2	0.21
86	Brain (dog)	"	50	5.3	1.5	3.8	2.4	1.4	0.07	0.15	64.73		0.25	0.2	0.175
87	Brain (dog)	"	50	4.8	1.25	3.5	2.2	1.3	0.07	0.15	62.23			0.2	0.15

Summarizing the results recorded in Table I, the following figures for the alcohol-extractable constituents are obtained:

2,081 grams of liver (70 different specimens from man) yielded:

(*a*) 175.50 grams of substances, equal to 8.4 per cent., which was divided into
    (*b*) 60.48 grams of ether-insoluble substances, equal to 2.9 per cent., and
    (*c*) 112.87 grams of ether-soluble substances, equal to 5.4 per cent., of which
        (*d*) 58.91 grams were aceton-insoluble, equal to 2.8 per cent., and
        (*e*) 61.24 grams were aceton-soluble, equal to 2.9 per cent.

363 grams of animal liver (four rabbits, five guinea pigs, three dogs) yielded:

(*a*) 26.65 grams of substances, equal to 7.3 per cent., which was divided into
    (*b*) 9.25 grams of ether-insoluble substances, equal to 2.5 per cent., and
    (*c*) 17.40 grams of ether-soluble substances, equal to 4.8 per cent., of which
        (*d*) 11.59 grams were aceton-insoluble, equal to 3.2 per cent., and
        (*e*) 6.02 grams were aceton-soluble, equal to 1.6 per cent.

Fluctuations in the amounts of the entire extracts and also in those of different fractions were considerable in different specimens of human livers. The maximum and minimum were as follows:

	Maximum.	Minimum.
Entire extract before fractionation	16.0 per cent. (No. 64)	4.0 per cent. (No. 38)
Ether-insoluble substances	10.0 per cent. (No. 64)	0.8 per cent. (No. 2)
Aceton-insoluble substances	4.5 per cent. (No. 17)	1.0 per cent. (No. 64)
Aceton-soluble substances	8.0 per cent. (No. 67)	0.8 per cent. (No. 51)

The extracts from animal livers showed the following relations:

	Maximum.	Minimum.
Entire extract before fractionation.	9.0 per cent. (No. 74)	4.8 per cent. (No. 77)
Ether-insoluble substances	3.0 per cent. (No. 74)	1.7 per cent. (No. 73)
Aceton-insoluble substances	4.0 per cent. (No. 79)	2.0 per cent. (No. 77)
Aceton-soluble substances	3.0 per cent. (No. 72)	0.8 per cent. (No. 77)

The weight of substances extractable by alcohol from different livers varies greatly, the variations being greater in extracts from human than in those from animal livers. This striking difference was probably due to the fact that the human specimens were pathological and had undergone a more pronounced alteration after death, the main one, no doubt, being a rapid autolysis.

The ether-insoluble substances from human livers showed the widest range of variation, the minimum per cent. being less than one-twelfth of the maximum. In the case of animals the minimum per cent. of the ether-insoluble substance varied from the maximum by less than one-half.

The variation in the amounts of the aceton-insoluble fraction was not so marked as in that of the ether-insoluble fraction, the maximum per cent. being

only four and a half times the minimum. The liver from a patient who had had severe anemia yielded the smallest aceton-insoluble fraction. The variation in the aceton-insoluble fraction of animal livers was small. Finally, the aceton-soluble fraction was seen to vary greatly, especially in human livers, where the maximum was ten times the minimum.

This great variation, according to Leathes, who studied the metabolism of fats with special reference to the liver in man and animals, was to be expected. It is produced through the transportation of stored fats (adipose) to the liver, in various pathological conditions, in order to convert the inert fats into active fats by desaturation of double carbon bonds. Not pathological conditions only, but physiological ones also, are capable of producing considerable fluctuations in this fraction.

It is noteworthy that the ether-insoluble fraction in autolysed liver of dog 83 is enormously large in comparison with the aceton-insoluble and aceton-soluble fraction which it yielded.

The aceton-insoluble fraction obtained from the hearts of guinea pigs is seen to be quite large, amounting to 6 per cent., while the aceton-soluble fraction is only 2.8 per cent., although in this percentage is included a certain amount of connective tissue fats.

The brains of guinea pigs and dogs yielded about 5 to 6 per cent. of aceton-insoluble fractions and 2 to 3 per cent. of aceton-soluble fractions, while in dogs and guinea pigs the ether-insoluble fraction in the brain extract reached 2 to 3 per cent.

The point of chief interest is the consideration of the extracts from the point of view of their biological behavior. In Table I the quantities of the aceton-insoluble and aceton-soluble fractions of each extract are recorded for hemolytic, anticomplementary, and antigenic activity. The blank spaces in the three columns signify that there was no action when a maximum dose of 0.4 cubic centimeter of each emulsion was used.

Aceton-soluble Fraction.—The majority of the emulsions of the aceton-soluble fraction were hemolytic. Of seventy specimens fifty-four (nearly 70 per cent.) showed this activity. Emulsion No. 41 was the strongest, 0.01 cubic centimeter causing complete hemolysis. In the case of the weakest emulsion, 0.25 cubic centimeter was required to bring about complete hemolysis.

The hemolytic activity did not, as one might suppose, vary with the acidity of the emulsion. In spite of being acid, certain specimens exerted no hemolytic activity. The nature of the acid present is very important, for Noguchi showed some years ago that the hemolytic activity of unsaturated fatty acids is nearly ten times stronger than the normal or saturated fatty acids. It is probable,

therefore, that the emulsions that here proved so strongly hemolytic contained a relatively large quantity of the unsaturated fatty acids. This point, however, requires further study.

The biological evidence for attributing hemolytic activity in some instances to the presence of large quantities of unsaturated fatty acids is, to a certain extent, supported by the results of Leathes, who has shown, by testing the iodine value of various liver extracts, that the quantities of saturated and unsaturated fatty acids in different specimens vary greatly.

In the aceton-soluble fraction, twenty extracts (28 per cent.) showed anticomplementary properties. This activity was manifested by quantities that varied from 0.03 to 0.5 cubic centimeter, the usual quantities being 0.1 to 0.2 cubic centimeter.

Antigenic Properties.—Of seventy specimens thirty-four (about 50 per cent.) manifested antigenic properties. In five specimens the antigenic activity was the only one observed. In the remaining ones, however, it was associated with other properties; in six, with an anticomplementary action; in sixteen, with a hemolytic property; and in seven with both hemolytic and anticomplementary properties.

It should be understood that those emulsions which possessed either hemolytic or anticomplementary properties, or both, were examined for their antigenic property in the quantities which were too small to manifest a hemolytic or anticomplementary action. The emulsions which were antigenic without the other two properties were generally weak; that is, they were active when employed in doses of 0.15, 0.2 and 0.3 cubic centimeter, and were very weak when compared with the aceton-insoluble fractions which will be mentioned below.

The results obtained with the aceton-soluble fraction of *animal livers* are similar to those obtained with the human livers, except that the presence of the anticomplementary property was more frequent in the animal specimens. In examining thirteen livers the anticomplementary property was found in ten (77 per cent.), the hemolytic property in eight (66 per cent.), and the antigenic property in seven (53 per cent.). Every emulsion showed either the hemolytic or the anticomplementary property, or both. Every specimen was acid.

The aceton-soluble fractions of the hearts and brains of guinea-pigs and dogs were in all respects comparable to those of the animal livers just described.

Aceton-insoluble Fraction.—In considering the results obtained with the aceton-insoluble fraction, one sees that the column for the records of the hemolytic property is, with one exception (No. 41) quite blank. In the exceptional case the figures 0.3 cubic centimeter are recorded. This means that sixty-nine out of seventy different emulsions had no hemolytic action when used in the large dose of 0.4 cubic centimeter. Emulsion No. 41 was hemolytic in the dose of 0.3 cubic centimeter. In the corresponding column of the seventy aceton-soluble fractions, fifty-four (70 per cent.) show hemolysis. The absence of the hemolytic activity is characteristic of the aceton-insoluble fraction, while the presence of this activity is characteristic of the aceton-soluble fraction. In the seventy specimens examined, the anticomplementary property was present in twenty-four (34 per cent.). It was, therefore, somewhat more frequently present here than in the aceton-soluble fraction. The anticomplementary property may be present or absent in the aceton-soluble or in the aceton-insoluble fractions derived from the same liver. Its presence in one fraction is, however, quite independent of its presence in the other.

Concerning the aceton-insoluble fractions, the following may be stated: the antigenic property was present in sixty-six liver fractions (94 per cent.). It was absent or extremely weak in three fatty livers, and in the fourth, from a case of tuberculosis, the extract could not be used on account of its hemolytic activity.

Of the sixty-six preparations that possessed an antigenic property, twenty-four (34 per cent.) had an auxiliary anticomplementary action, while forty-two (60 per cent.) were entirely devoid of this.

2. THE RELATION BETWEEN THE ANTIGENIC AND ANTICOMPLEMEN-TARY PROPERTIES OF THE ACETON-INSOLUBLE FRACTIONS.

Table II shows the anticomplementary property of twenty-four emulsions. These emulsions can not be recommended for the quantitative estimation of complement fixation, although some of them could be used for routine diagnosis for the reason that the amount of the extract necessary to exhibit the antigenic property, differed sufficiently from that which would mani-

TABLE II.

Antigenic titers.	Anticomplementary titers.	Number of preparations of each group.	Ratio of the antigenic to the anticomplementary titers	Remarks.
0.02 c.c.	0.1 c.c.	1	1: 5	Unsuitable for quantitative work.
	0.2 c.c.	4	1:10	Unsuitable for quantitative work.
0.03 c.c.	0.2 c.c.	4	1: 6.6	Unsuitable for quantitative work.
	0.4 c.c.	2	1:13.3	Unsuitable for quantitative work.
0.04 c.c.	0.1 c.c.	1	1: 2.5	Unsuitable.
	0.15 c.c.	1	1: 3.75	Unsuitable.
	0.2 c.c.	5	1: 5	Unsuitable for quantitative work.
	0.4 c.c.	1	1:10	Unsuitable for quantitative work.
0.5 c.c.	0.2 c.c.	4	1:4	Unsuitable.
	0.4 c.c.	1	1:8	Unsuitable for quantitative work.
		24		Unsuitable 6
				Conditional 18
				24

fest a disturbing anticomplementary action. The fact that in the fixation test *two units* of complement and of amboceptor are prescribed, renders negligible the possibility of an interference by the anticomplementary property. The anticomplementary effect no longer disturbs when the quantity corresponding to one-third of its titer is tested against *one unit* of complement. Where the relation of the antigenic titer to the anticomplementary titer is as close as 1 : 2.5, 1 : 3.75, or 1 : 4, the emulsion should not be employed even for routine work. It may be mentioned that Wassermann and his pupils determined the minimum quantity of liver extract in which the anticomplementary action could be detected, and they recommended the use of half of this quantity in carrying out the Wassermann reaction.

This principle, while safeguarding the reaction from a non-specific inhibition due to the anticomplementary property, leaves the standard of the antigenic strength of the extract undefined. The quantity they use may contain one antigenic unit, or it may contain several. If the antigenic unit is determined with a strongly positive serum (e. g., six antibody units), this unit is no longer able to detect weaker reactions (e. g., one or two antibody units), while it is easy to do so if the antigenic unit is adjusted to weaker sera.

The results obtained with animal livers agree, in general, with those obtained with human livers. None of the emulsions were hemolytic. On the other hand, the anticomplementary property was present more frequently. All except one emulsion (No. 76) could be used for routine diagnostic work. The antigenic titers were somewhat inferior to those found with the human specimens. The autolyzed liver of dog 83, although having a high antigenic titer, yielded an emulsion free from hemolytic or anticomplementary

properties. The emulsion prepared from guinea-pig hearts possessed a weak anticomplementary and a strong antigenic property.

The biological characteristics of the aceton-insoluble fraction of dog and guinea-pig brains were quite different from those of the liver extracts. While devoid of a hemolytic property, these emulsions were anticomplementary and possessed a slight antigenic property. They are, therefore, unfit for use in the complement fixation test.

The estimation of the iodine absorption in these preparations seems to indicate that there is a certain relationship between the iodine value and the antigenic activity. With few exceptions all preparations with a high antigenic value possessed a high iodine value, and the preparations without antigenic value gave the lowest iodine value. The brain preparations, which usually had a higher iodine value, did not conform to the above generalization, as they had only feeble antigenic values. At present, no explanation for this difference can be given. It is, however, not improbable that the unsaturated carbon bonds in the brain lipoids are situated in different positions from those in the antigenically active lipoids of the liver or heart. Further experimental work, however, is required to determine this point.

3. THE ASSOCIATION BETWEEN CLINICAL CONDITIONS AND THE ANTIGENIC AND IODINE VALUES OF THE ACETON-INSOLUBLE FRACTIONS.

In Table III the clinical diagnosis of the patients from whom the livers were obtained is shown together with an estimation of the comparative frequency and distribution of the antigenic and anticomplementary properties in the aceton-insoluble fractions of these livers. At the bottom of the table we have recorded the figures showing the average iodine values for different antigenic titers and also the maximum and the minimum for each antigenic titer.

4. THE ASSOCIATION OF PATHOLOGICAL CONDITIONS OF THE LIVER WITH THE ANTIGENIC VALUE OF THE ACETON-INSOLUBLE FRACTIONS.

An examination of Table IV does not enable us to discover a definite relation between the antigenic value and the pathological conditions of the livers, except that the fatty livers in no instance yielded a serviceable extract. It is probable that the fatty acid radicals, constituting the lipoids of a fatty liver, belong chiefly to the saturated varieties. The low iodine values of these lipoids are in favor of this hypothesis.

TABLE III.

Mode of Distribution of Different Preparations (Aceton-insoluble Fraction) Arranged according to the Clinical Diagnosis.

	Number of preparations.	Antigenic titer of 0.02 c.c.		Antigenic titer of 0.03 c.c.		Antigenic titer of 0.04 c.c.		Antigenic titer of 0.05 c.c.		Antigenic titer of 0.07 c.c.		Antigenic titer of 0.1 c.c.		Preparations without antigenic value.
		Not anti-complementary.	Anticom-plementary.	Not anti-complementary.	Anticom-plementary.	Not anti-complementary.	Anticom-plementary.	Not anti-complementary.	Anticom-plementary.	Not anti-complementary.	Anticom-plementary.	Not anti-complementary.	Anticom-plementary.	
General paralysis	16	2		1	1	3	3	2	2					2
Senile dementia	2	1		1		2			2					1
Senile psychosis	10	2		1	1	3	1	3	1					
Dementia precox	8	1			1			1	1					
Epileptic psychosis	1													
Manic depressive insanity	1						1							
Imbecility	1									1				
Cerebral hemorrhage	1													
Pulmonary tuberculosis	4				1	1	1	1	1					1
Tubercular meningitis	2		1		1									
Tubercular peritonitis	1		1											
Erysipelas	2			1				1						
Acute peritonitis	4	2						1						
Pneumonia	4				1	2								
Acute enteritis	1		1											
Gastric carcinoma	1	1												
Chronic alcoholism	1		1				1							
Cirrhosis of liver	1							1						
Pulmonary thrombosis	1	1						1						
Cardiac insufficiency	1													
Malformation of heart	1				1									
Grave anemia	1													
Uremia	1	1												
Cerebral abscess	1		1			1								
Premature child?	1	1												
Fetus ?	1	1												
Pulmonary infarct	1	1												

	Number of preparations	Antigenic titer of 0.02 c.c.		Antigenic titer of 0.03 c.c.		Antigenic titer of 0.04 c.c.		Antigenic titer of 0.05 c.c.		Antigenic titer of 0.07 c.c.		Antigenic titer of 0.1 c.c.		Preparations without antigenic value.
		Not anti-complementary.	Anticomplementary.	Not anti-complementary.	Anticomplementary.	Not anti-complementary.	Anticomplementary.	Not anti-complementary.	Anticomplementary.	Not anti-complementary.	Anticomplementary.	Not anti-complementary.	Anticomplementary.	
Rabbit liver	4	1			1		2		1					
Guinea pig liver	5		4		2	2	3	1						
Dog liver	3													
Dog liver (autolyzed)	1													
Total	83	14	5	4	9	14	12	12	6	1				4
Anticomplementary in doses of														
0.1 c.c.					1		2		5					
0.15 c.c.			1		6		3		1					
0.2 c.c.			4		2		7		1					
0.4 c.c.							1							
Average iodine value		55.43		48.50		44.50		40.17		34.35		28.26		
Highest iodine value		69.85		56.54		58.36		49.47				31.75		
Lowest iodine value		47.62		44.45		40.00		34.47				21.94		

TABLE IV.

Frequency and distribution of different preparations for the biological properties. — Distribution of biological properties among the pathological conditions.

Liver.	0.02 c.c. Not anticomplementary.	0.02 c.c. Anticomplementary.	0.03 c.c. Not anticomplementary.	0.03 c.c. Anticomplementary.	0.04 c.c. Not anticomplementary.	0.04 c.c. Anticomplementary.	0.05 c.c. Not anticomplementary.	0.05 c.c. Anticomplementary.	0.07 c.c. Not anticomplementary.	0.07 c.c. Anticomplementary.	Preparations without antigenic value in dose of 0.1 c.c.	Preparations without anticomplementary action.	Preparations with anticomplementary action.	Preparations without antigenic value.	Total.
Human.															
Normal	9	5	3	5	8	4	6	4			1	26	18	1	45
Passive congestion			1	1	5	4	3	1				9	6		15
Anemic	1						1					2			2
Soft and friable	1											1			1
Cirrhotic	2											2			2
Fatty									1		3	1		3	4
82 hr. after death							1					1			1
Guinea pig, rabbit, dog.															
Anemic (bled)						2							2		2
Autolyzed	1								•			2			2
Anemic (bled)				1		3							4		4
Anemic (bled)				2	2			1				2	3		5
Total	14	5	4	9	15	13	11	6	1		4	46	33	4	83

TABLE V.

Frequency and Distribution of Different Preparations (Aceton-insoluble Fraction) Arranged according to Age of Patients.

	Distribution of different preparations with reference to biological properties.											Frequency of biological activity for different ages.			
	Preparations with the antigenic titers of										Preparations without antigenic value.	Preparations without anticomplementary action.	Preparations with anticomplementary action.	Preparations without antigenic value.	Total.
	0.02 c.c.		0.03 c.c.		0.04 c.c.		0.05 c.c.		0.07 c.c.						
Age	Not anticomplementary.	Anticomplementary.	Not anticomplementary.	Anticomplementary.	Not anticomplementary.	Anticomplementary.	Not anticomplementary.	Anticomplementary.	Not anticomplementary.	Anticomplementary.					
Under 1 yr		3	1	1	1		1		1				4		8
1 to 10 yr			1	2								1	2		3
11 to 20 yr				1							1		1	1	2
21 to 40 yr	4	2	2	2	2	1	3	1				11	6		17
41 to 60 yr	3				7	5	4	3			2	14	8	2	24
61 to 80 yr	5				3	1	3	1			1	11	2	1	14
over 80 yr	1					1						1	1		2
Total	13	5	4	6	13	8	11	5	1		4	42	24	4	70

5. THE ASSOCIATION BETWEEN THE AGE AND THE ANTIGENIC VALUE OF THE ACETON-INSOLUBLE FRACTIONS.

The foregoing analysis seems to show, in a general way, that the livers from individuals under twelve months of age yield lipoids of a higher antigenic value. With this exception no definite relation between the age and the antigenic power could be established. We suggest, however, that the possibilities of encountering pathological conditions leading to fatty degeneration of the liver are greater in adults. From this it would follow that the livers of older people are more likely to yield unserviceable lipoid fractions. Here the age is no more than a predisposition, and not the direct cause. The determining factor is, therefore, the pathological condition itself. The fact is worth recording that one of us (Noguchi) has on two occasions observed that, in spite of its normal appearance, the liver of a tubercular patient yielded a fraction which was distinctly hemolytic and only feebly antigenic.

STANDARD ANTIGEN FOR NOGUCHI'S METHOD.

A standard antigen for Noguchi's method for the diagnosis of syphilis may be prepared as follows: as a stock solution make an ethereal or alcoholic 3 per cent. solution of an aceton-insoluble fraction of the liver or heart. From this an emulsion is made by shaking one volume of the stock solution with nine volumes of a 0.9 per cent. sodium chloride solution. This emulsion is tested for its properties. If it is hemolytic or anticomplementary, in the dose of 0.4 cubic centimeter, it is unsuitable. When the emulsion is found to be non-hemolytic and non-anticomplementary, it is tested for its antigenic strength. If it produces complete inhibition of hemolysis with one unit of syphilitic antibody in doses of 0.02 cubic centimeter or less, it is suitable. In the fixation test 0.1 cubic centimeter of such an emulsion is to be used, thus employing more than five times the minimal antigen dose. So far as our experience goes, the use of several antigen doses does not cause a non-specific fixation and is not unduly sensitive.

The stock solution of antigen retains its properties for a long time. A solution prepared over a year ago has apparently undergone no change.

SUMMARY.

The liver tissues of man and certain animals (dogs, rabbits, guinea pigs, etc.) yield, upon alcoholic extraction, various substances which may be divided by their physical and chemical properties into several groups. While many substances are present in the alcoholic extract, the ones possessing antigenic properties are comparatively few. The latter are responsible for the antigenic properties exhibited by the whole alcoholic extract. The substances extracted with alcohol were fractionated into the following four groups.

(*a*) *Substances Insoluble in Ether and Hot Alcohol.*—These are chiefly proteins and salts. The proteins are probably the minute particles of larger molecules held in apparent suspension in alcohol until all other substances are removed. The water extracted from the tissues and admixed with alcohol is also an essential factor in extracting these particles in an alcoholic solution. The salts present are the usual physiological constituents of the liver, notably, sodium chloride. The quantity of these substances extracted with alcohol varies greatly with different specimens. Biologically considered, they are neither markedly hemolytic nor anticomplementary and possess no antigenic property for the Wassermann reaction. It is important, however, to note that the proteins bind complement when mixed with certain active human sera. For this reason a preparation of antigen containing this group of substances is unsuitable for use in combination with an *active* serum, and should, therefore, be rejected.

(*b*) *Substances Insoluble in Ether and Soluble in Hot Alcohol.*— This group contains soaps, cleavage products of proteins, and small amounts of the bile salts. Soaps and bile salts are very strongly hemolytic and are absolutely unfit for use as antigen. Moreover, their antigenic properties are very slight. It is best to eliminate this group of substances from the preparation of antigen. The quantity of the substances of this group extracted from different specimens of tissue is very variable.

(*c*) *Substances Soluble in Ether, Alcohol, and Aceton.*—In this group are found varying amounts of fatty acids, both saturated and unsaturated, some neutral fats, cholesterin and many unidentified lipoidal bodies. This group causes either hemolysis or inhibition of hemolysis. In other words, it is anticomplementary as well as hemolytic in the majority of preparations. At the same time, in some preparations it is, to a certain extent, antigenic. This great variation in the amounts of these substances in given extracts renders their presence in the antigen preparation undesirable. It is not denied, however, that, when added in adequate quantities, some of these substances may improve the activity of the antigenic lipoids.

(*d*) *Substances Insoluble in Aceton.*—This group of substances consists of phosphatids. The best known among them is, of course,

lecithin. Besides lecithin, however, there must be various other phosphatids present in this fraction. It will be noticed that the precipitate formed by mixing the ethereal solution with aceton contains a certain amount of lipoids insoluble in ether as well as in alcohol. Before the fractionation in aceton, all lipoids were soluble in ether or ethyl alcohol. Further analytical work on the nature of the phosphatids contained in this fraction is necessary. This fraction, in general, is more constant in amount in the various liver extracts. Biologically considered, it is the most important. It is usually nonhemolytic, frequently anticomplementary, but much more strongly antigenic than the other fractions. The antigenic strength varies with different preparations, being almost absent in the extracts derived from fatty livers. An aceton insoluble fraction may be strongly antigenic without any other auxiliary effects, or may be accompanied by an anticomplementary property. This fraction does not cause the so-called non-specific reaction with an active human serum. For these reasons it is recommended (as Noguchi has repeatedly done before) that the antigen should be selected from the aceton-insoluble fractions.

Iodine Value in Relation to Antigenic Value.—Speaking generally of the aceton-insoluble fraction, a high antigenic value is associated with a high iodine value of lipoids obtained from the liver and heart. This rule does not, however, apply to the corresponding fraction from the brain.

Standard Antigen.—The preparation of a standard antigen for Noguchi's method for the diagnosis of syphilis is given just before the summary. The directions will enable one to make a stock solution, from which, at any time, a suitable antigenic emulsion may be obtained.

VARIATIONS IN THE COMPLEMENT ACTIVITY AND FIXABILITY OF GUINEA PIG SERUM.[1]

By HIDEYO NOGUCHI and J. BRONFENBRENNER.

(From the Laboratories of the Rockefeller Institute for Medical Research, New York.)

Some time ago one of us (Noguchi) encountered a peculiar action in goat serum. This serum, while fresh and active, remained undeviated by the Wassermann reaction or by any combination of specific antigen and antibody. It was natural, therefore, to inquire whether a similar action might not be found in the sera of other species of animals or in the sera of individuals of a species in which the complement is generally capable of deviation.

Our present paper concerns itself with the quantitative determination of the complementary activity of various sera and their behavior towards the complement deviation phenomenon.

The most important and most widely studied of all complements is that of the guinea pig. Although there are some observations bearing upon the relative complementary activity of different specimens of this serum, there is no work in which quantitative estimations of complementary activity of a large number of specimens with simultaneous determinations on the fixability of each has been carried out. This has been done in our present studies.

We have also studied the complementary activity of the sera of several other species, such as beef, sheep, goat, pig, dog, cat, rabbit, rat, chicken, etc.[2] Some of these sera were tested for their fixability, our purpose being to see whether the complements of any of these could be used for the fixation test.

METHOD OF STUDY.

1. *The Titration of the Activity of Complement.*—The amboceptor derived from rabbits immunized with washed human corpuscles was used in all the

[1] Received for publication, September 21, 1910.
[2] The results with these sera will be published separately.

TABLE I.

Serial number of guinea pig sera.	Complement activity.							Fixability (20-hour-old serum).		
	Serum left in contact with clot for 20 hours (o° C.).			Serum left in contact with clot for 46 hours (o° C.).			Activity after 46 hours.	Amount of guinea pig serum fixed.	Number of complement units fixed.	Remarks on fixability.
	100 per cent. Hemolysis.	50 per cent. Hemolysis.	25 per cent. Hemolysis.	100 per cent. Hemolysis.	50 per cent. Hemolysis.	25 per cent. Hemolysis.				
1	0.025	0.012	0.006	0.025	0.012	0.006		0.135	5.4	Marked.
2	0.022	0.01	0.005	0.015	0.006	0.0035		0.12	5.45	Marked.
3	0.022	0.01	0.005	0.025	0.012	0.0055		0.09	4.09	Normal.
4	0.022	0.01	0.005	0.015	0.007	0.0035	Increased	0.09	4.09	Normal.
5	0.022	0.01	0.005	0.015	0.007	0.004		0.09	4.09	Normal.
6	0.022	0.01	0.005	0.022	0.009	0.005	Increased.	0.147	6.68	High.
7	0.018	0.008	0.004	0.04	0.02	0.01	Increased.	0.085	4.72	Normal.
8	0.018	0.008	0.005	0.022	0.01	0.005	Marked decrease.	0.09	5.	Normal.
9	0.022	0.009	0.005	0.022	0.01	0.005	Decreased.	0.09	4.09	Normal.
10	0.015	0.0065	0.004	0.013	0.006	0.003		0.09	6.	High.
11	0.017	0.0075	0.004	0.015	0.007	0.0035		0.09	5.3	Marked,
12	0.015	0.007	0.004	0.017	0.0075	0.0035		0.12	6.	High.
13	0.022	0.01	0.006	0.025	0.012	0.005		0.09	5.83	High.
14	0.02	0.008	0.004	0.015	0.006	0.003	Slight increase.	0.06	4.5	Normal.
15	0.022	0.01	0.006	0.015	0.006	0.003	Increased.	0.09	2.54	Highly refractory,
16	0.025	0.012	0.006	0.02	0.01	0.005	Slight increase.	0.09	3.6	Markedly refractory.
17	0.025	0.012	0.006	0.025	0.0121	0.006		0.15	6.	High.
18	0.02	0.01	0.005	0.018	0.008	0.004		0.09	4.5	Normal
19	0.022	0.01	0.005	0.02	0.01	0.005	Slight decrease.	0.085	3.86	Markedly refractory.
20	0.02	0.008	0.004	0.022	0.009	0.005		0.14	7.	High.
21	0.02	0.008	0.004	0.02	0.008	0.004	Slight increase.	0.085	4.25	Normal.
22	0.018	0.008	0.004	0.018	0.0065	0.004	Slight increase.	0.09	4.5	Normal.
23	0.02	0.008	0.004	0.015	0.006	0.003	Slight decrease.	0.09	5.	Normal.
24	0.02	0.008	0.004	0.022	0.01	0.006	Increased.	0.09	4.5	Normal.
25	0.02	0.008	0.004	0.015	0.006	0.0035		0.09	4.5	Normal.
26	0.022	0.009	0.005	0.015	0.006	0.003	Marked increase.	0.03	1.66	Highly refractory.

Serial number of guinea pig sera.	Complement activity.							Fixability (20-hour-old serum).		
	Serum left in contact with clot for 20 hours (0° C.).			Serum left in contact with clot for 46 hours (0° C.).			Activity after 46 hours.	Amount of guinea pig serum fixed.	Number of complement units fixed.	Remarks on fixability.
	Hemolysis 100 per cent.	Hemolysis 50 per cent.	Hemolysis 25 per cent.	Hemolysis 100 per cent.	Hemolysis 50 per cent.	Hemolysis 25 per cent.				
27	0.02	0.008	0.004	0.02	0.008	0.0045	Slight increase.	0.09	4.5	Normal.
28	0.018	0.0075	0.004	0.015	0.006	0.004	Slight increase.	0.09	5.	Normal.
29	0.018	0.0075	0.004	0.015	0.006	0.0035	Increased.	0.09	5.	Normal.
30	0.035	0.015	0.0075	0.022	0.0075	0.004	Marked increase.	0.09	2.57	Highly refractory.
31	0.025	0.01	0.0045	0.015	0.006	0.003	Slight decrease.	0.15	3.6	Markedly refractory.
32	0.02	0.008	0.004	0.022	0.01	0.006	Marked decrease.	0.24	7.5	High.
33	0.025	0.01	0.005	0.06	0.03	0.015	Increase.	0.	9.6	Unusual.
34	0.025	0.01	0.004	0.018	0.008	0.005		0.075	0.	Absolutely refractory.
35	0.025	0.01	0.004	0.022	0.0085	0.0045	Slight increase.	0.09	3.	Highly refractory.
36	0.02	0.008	0.004	0.022	0.009	0.005	Slight decrease.	0.105	4.5	Normal.
37	0.018	0.0075	0.004	0.018	0.007	0.0035	Marked decrease.	0.12	5.8	Marked.
38	0.027	0.012	0.005	0.04	0.02	0.01	Marked increase.	0.12	4.44	Normal.
39	0.025	0.01	0.004	0.018	0.008	0.005	Slight increase.	0.09	4.8	Normal.
40	0.025	0.01	0.005	0.022	0.01	0.005	Slight increase.	0.09	3.6	Markedly refractory.
41	0.027	0.012	0.004	0.017	0.007	0.004	Marked increase.		3.33	Markedly refractory.
Averages	0.0216			0.0209				0.098	4.64	

hemolytic experiments. In addition to this, another amboceptor derived from a goat similarly immunized was employed in certain experiments recorded in the latter part of this paper. Unless otherwise stated, however, rabbit amboceptor is referred to.

The amboceptor was used in constant doses. For each tube 0.1 c.c. of a 10 per cent. suspension of washed human corpuscles was used. The complement to be titrated was added to a series of tubes in graduated quantities. The total volume of fluid in each tube was brought up to 1 c.c. by means of the addition of 0.9 per cent. salt solution. The tubes were placed in a water thermostat and incubated for two hours at 37° C.

2. *The Determination of the Fixability of Complement.*—In determining the fixation of complement, the system used was the one introduced by Noguchi for the diagnosis of syphilis. The amount of antihuman amboceptor (rabbit) was uniformly two units for each tube The quantity of washed corpuscles used was 0.1 c.c. of a 10 per cent. suspension. The quantity of complement varied according to the titers of the different sera. The amount of antigen emulsion was 0.1 c.c., which contained about four antigenic units. It was made from the aceton-insoluble fraction of lipoids from a human liver, according to Noguchi's method.[3] In doses of 0.45 c.c. it had no anticomplementary action. The serum was a mixture from several untreated cases of secondary syphilis and was inactivated before use. It had no inherent anticomplementary property in doses of 0.2 c.c., and in the fixation experiments the quantity employed was only onetenth of this.

The determination of the amount of fixed complement was made by reading, according to the colorimetric method of Madsen, the degree of hemolysis present in a tube where an incomplete fixation had taken place, and then calculating the approximate amount of complement still free. As the exact amount of complement originally employed is known, it is an easy matter to calculate the amount of complement which had been fixed.

The results obtained are given in Table I. In this series of experiments forty-one guinea pigs were examined at the same time.

In studying the results summarized in Table I, several interesting facts may be noticed. It was rather unexpected to find that the complementary activity increased more or less after the sera remained in contact with the clot for forty-six hours, when compared with the activity of the same sera left in contact with the clot for twenty-hours after the bleeding. A few sera, however, became somewhat weaker under the same circumstances. The following analysis gives the results that were obtained.

The average titers for the first (twenty hours) and second (forty-six hours) determinations are nearly the same, being 0.0216 cubic centimeter for the first, and 0.0209 cubic centimeter for the

[3] Serum Diagnosis of Syphilis, 1st edition, Philadelphia, 1910, p. 71.

Comparable titers.	Number of sera examined.	
	Determination after 20 hours.	Determination after 46 hours
0.013 c.c.	0	1
0.015 c.c.	2	12
0.017 c.c.	1	2
0.018 c.c.	6	5
0.02 c.c.	10	4
0.022 c.c.	10	10
0.025 c.c.	9	4
0.027 c.c.	2	0
0.03 c.c.	1	0
0.035 c.c.	0	0
0.04 c.c.	0	2
0.06 c.c.	0	1
	41	41

second. It will be noticed, also, that the differences between the highest and the lowest titers are much greater with the second determination. To insure greater uniformity in action, it is preferable to employ sera which have been left in contact with the clot for about twenty-four hours.

The titers of these forty-one sera are not absolute; they can be made higher by employing a larger quantity of amboceptor. We sought not absolute, but relative titers in our present experiments, and the following chart shows the frequency with which the same titers were obtained with different sera.

CHART I.

Titers.
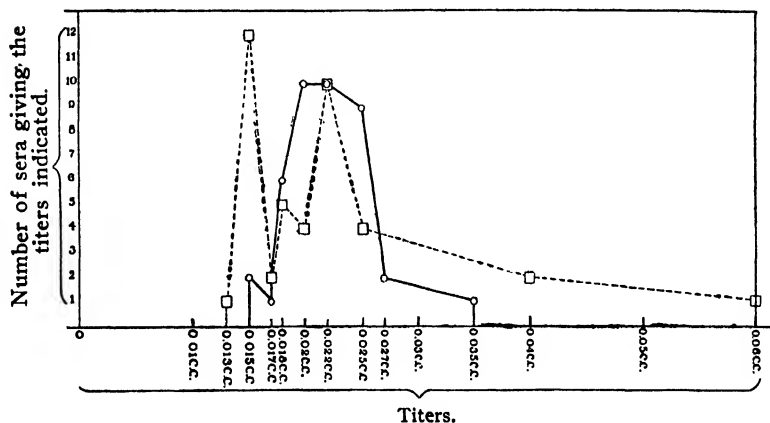
-O- Determination for the sera that remained in contact with the clot for 20 hours (0°C.)

-□- Determination for the sera that remained in contact with the clot for 46 hours (0°.C)

Let us now consider the relative susceptibility of different sera to the fixing phenomenon in the syphilis reaction.   Here we have two

Amount of guinea pig serum fixed.	Number of specimens having the titers indicated.	Amount of guinea pig serum fixed.	Number of specimens having the titers indicated.
0.0 (non-fixable)	I	0.12 c.c.	4
0.03 c.c.	I	0.135 c.c.	I
0.06 c.c.	I	0.14 c.c.	I
0.075 c.c.	I	0.147 c.c.	I
0.085 c.c.	3	0.15 c.c.	I
0.09 c.c.	24	0.24 c.c.	I
0.105 c.c.	I		
			4I

CHART 2.

Amounts of serum fixed.

problems. The first is to determine the absolute quantities of different sera fixed by given constant amounts of syphilitic serum and antigen; and the second is to determine the number of units of complement that have disappeared. The absolute amounts of sera fixed under the same conditions vary considerably with different specimens, but the variation in the majority of the specimens is slight.

The contrast between the extremes in the fixation of guinea pig serum is most striking. The non-fixable serum (No. 34) showed no abnormal complementary activity, and the highest fixable serum (No. 33) had also the usual titer of activity, although the latter deteriorated with an unusual rapidity during forty-six hours. Another specimen (No. 38) had also shown a rapid deterioration, but there was no abnormality in its fixability.

Taking up the same subject from the standpoint of unity of complementary activity, the following statistics were obtained. In grouping the units, the fractions intermediate between two standard divisions were included in the nearest division. Several arbitrary zones of fixability may be created (see below) in order to enable us to gain a better idea of the occurrence of various degrees of fixability among the sera thus examined. In the zone of normal fixability

Number of units of complement fixed.		
0.	1	Zone of non-fixability, 1 specimen (2.4 per cent.).
1.5	1	
2.	0	
2.5	2	Zone of inferior fixability, 8 specimens (19.5 per cent.).
3.	1	
3.5	4	
4.	6	
4.5	9	Zone of normal fixability, 20 specimens (48.7 per cent.).
5.	5	
5.5	3	
6.	5	
6.5	1	
7.	1	
7.5	1	Zone of superfixability, 12 specimens (29.2 per cent.).
8.	0	
8.5	0	
9.	0	
9.5	1	
	41	

are included the specimens which varied from the average by 0.5 of a unit in either direction.

CHART 3.

Units of complement fixed.
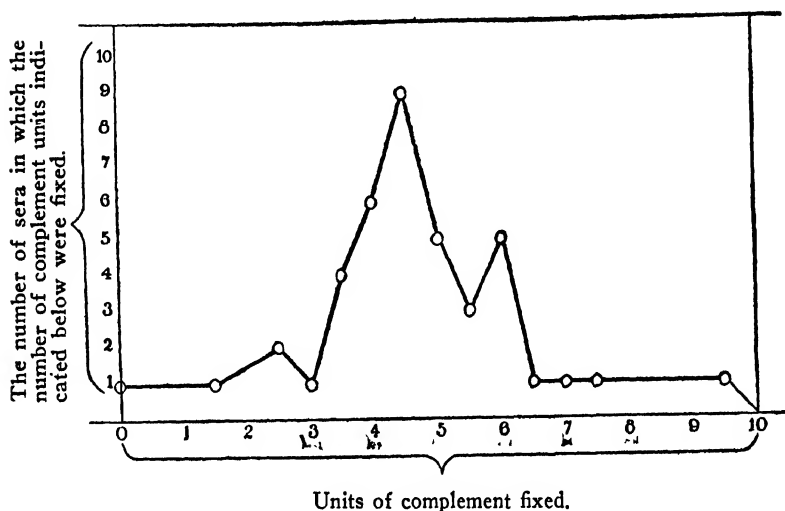
The average amount of serum fixed was 0.098 cubic centimeter (see Table I, p. 71) and the average number of units fixed was 4.64 (see Table I, p. 71). In fact, the majority of sera possessed a susceptibility to fixation not far removed from the average.

SUMMARY.

The following conclusions may be drawn from the foregoing series of experiments. The complementary activity varies within a definite limit in different specimens of guinea pig serum. With sera which stood in contact with the clot for twenty hours, the strongest and weakest were in the ratio of 0.015 cubic centimeter to 0.04 cubic centimeter. The former was 2.66 times stronger than the latter. The variation observed with the same series of sera after forty-six hours was still more striking. The strongest was 0.013 cubic centimeter, and the weakest, 0.06 cubic centimeter, that is, the former was 4.6 times stronger than the latter. These findings agree with those made by Massol and Grysez.[4] The variations

[4] Massol and Grysez, *Compt. rend Soc. de biol.,* 1910, lxvii, 588.

were not so marked with the majority of sera. It is noteworthy that a large number of the sera gained in the complementary activity when remaining in contact with the clot for forty-six hours, while some sera became weakened during the same length of time.

The amount of serum *fixed* by given constant quantities of syphilitic serum and antigen varies much more markedly than the variations in their *complementary* activity. One serum failed altogether to be fixed. On the other hand, one sample of serum was so easily fixable that 0.24 cubic centimeter (corresponding to 9.6 complement units of this specimen) disappeared, while the average quantity fixed was only 0.098 cubic centimeter (corresponding to 4.64 complement units). The normal standard of fixability was shown in about 50 per cent. of the specimens examined. If the zone of normal fixability is enlarged in both directions by one unit, the percentage of normal fixability would become 65.8. There is no definite relationship between the complementary activity and the fixability of a given specimen of guinea pig serum.

The facts derived from our present experiments, especially in regard to the exceptions in the fixative quality of this serum, demand the utmost precaution from those intending to employ it for diagnostic purposes, as, for example, in the Wassermann reaction. No quantitative work is possible with the complement fixation reaction unless the experimenter is capable of determining the fixability of the serum in use. One of us (Noguchi) has long realized this source of error, and in order to reduce it he has advised the employment of a mixture of sera from more than two guinea pigs.

THE COMPARATIVE MERITS OF VARIOUS COMPLE-
MENTS AND AMBOCEPTORS IN THE SERUM
DIAGNOSIS OF SYPHILIS

By HIDEYO NOGUCHI AND J. BRONFENBRENNER

THE COMPARATIVE MERITS OF VARIOUS COMPLE-
MENTS AND AMBOCEPTORS IN THE SERUM
DIAGNOSIS OF SYPHILIS.[1]

BY HIDEYO NOGUCHI AND J. BRONFENBRENNER.

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New York.*)

When using a hemolytic antihuman amboceptor produced in the
goat, one of us (Noguchi)[2] found that goat complement could not
be deviated or fixed by the precipitate formed by mixing an extract
of *Diplococcus intracellularis* (Weichselbaum) with antimeningo-
coccic serum (Flexner-Jobling), or by mixing sera of man or dif-
ferent animals with their specific precipitins (rabbit), or egg-white
with its specific precipitin. Under similar conditions, goat comple-
ment can not be deviated in Noguchi's method of the syphilis
reaction. The amboceptor from the goat seemed to have a
stronger affinity for the goat complement and liberated the comple-
ment after it had been fixed. Noguchi·has also repeatedly shown,
in the Bordet-Gengou phenomenon or in the syphilis reaction, that
rabbit complement is less firmly fixed than guinea pig complement.
As he was using a rabbit amboceptor, he could not determine
whether the difficulty in fixing this complement was due to the use
of an homologous amboceptor (from the same species) or to the
inferior fixing qualities of the complement itself. In order to de-
termine this point, it was necessary to prepare amboceptors from
different species of animals. The preceding problem, together with
others concerning the comparative merits of the complements of
various species of animals in the complement fixation phenomenon,
the Bordet-Gengou, and the Wassermann reactions, is considered
in this communication. The questions just outlined include the
determinations of the complementary activity of the sera of various

species of animals with homologous and heterologous amboceptors. They also bring out the relative capacities of different species of animals for producing hemolytic amboceptor for human erythrocytes. Apart from the scientific interest, we intended also to search for a suitable species of the larger domestic animals whose complement or amboceptor would yield results as reliable as those obtained with guinea pig complement and rabbit amboceptor. If this should be accomplished, an economy would result.

PREPARATION OF ANTIHUMAN AMBOCEPTOR.

In our experiments the washed human erythrocytes suspended in 0.9 per cent. salt solution, corresponding with the original volume of the blood, was used for immunizing the animals. The varieties of animals used and the quantities of erythrocytes injected are given below:

Dog, subcutaneous injection,	20, 30, 30, 30 c.c.
Cat, intraperitoneal injection,	5, 10, 15, 20 c.c.
Rabbit, intraperitoneal injection	5, 10, 15, 20 c.c.
Guinea pig, intraperitoneal injection,	2, 3, 5, 7 c.c.
Rat, intraperitoneal injection,	1, 2, 3, 3 c.c.
Chicken, intraperitoneal injection,	3, 4, 5, 5 c.c.
Goat,[1] subcutaneous injection.	

With the exception of the goat, more than two animals of each of the species were employed, and several of each of the smaller animals. Injections were made four to five days apart, and the serum was collected on the ninth day after the last injection. These amboceptoric sera were inactivated at 56° C. for thirty minutes. After inactivation, the strength of amboceptor of each of the above animals was determined by titration with homologous and heterologous complement. The complements employed were from dog, cat, rabbit, guinea pig, rat, chicken, goat, pig, sheep, and ox. Owing to the small quantity of blood that could be obtained from the immunized rats, the amboceptor from this animal could not be titrated to the extent of the others.

The amount of complement of each species used was uniformly 0.05 cubic centimeter for each tube. The erythrocytic suspension

[1] This goat was given about twenty subcutaneous injections.

TABLE 1.

	Rabbit amboceptor.				Guinea pig amboceptor.				Dog amboceptor.				Cat amboceptor.				Chicken amboceptor.			
	Complete hemolysis.	Moderate hemolysis.	Trace of hemolysis.	No hemolysis.	Complete hemolysis.	Moderate hemolysis.	Trace of hemolysis.	No hemolysis.	Complete hemolysis.	Moderate hemolysis.	Trace of hemolysis.	No hemolysis.	Complete hemolysis.	Moderate hemolysis.	Trace of hemolysis.	No hemolysis.	Complete hemolysis.	Moderate hemolysis.	Trace of hemolysis.	No hemolysis.
Rabbit complement.	**0.005**	**0.003**	**0.0007**	**0.0003**	0.0015	0.0007	0.00035	0.00015	0.0025	0.001	0.0005		0.004	0.0025	0.005					0.05
Guinea pig complement.	0.0025	0.0015	0.0005	0.0002	0.001	0.0005	0.0002	0.0001	0.025	0.015	0.001	0.0005	0.015	0.01	0.001	0.0005				0.05
Pig complement.	0.04	0.015	0.0025	0.001	0.025	0.015	0.001	0.0005	0.015	0.01	0.0005	0.0002	0.015	0.01	0.001	0.0005	0.08	0.03	0.005	0.002
Sheep complement.	0.05	0.025	0.005	0.0025	0.05	0.025	0.005	0.0025				0.05				0.05				0.05
Ox complement.	0.045	0.025	0.005	0.0007	0.04	0.02	0.005	0.0007	0.04	0.02	0.005	0.0007	0.025	0.015	0.0035	0.0007				0.05
Rat complement.	0.01	0.005	0.001	0.0005	0.01	0.005	0.0015	0.0005	0.025	0.025	0.005	0.002		0.04	0.025	0.015				0.05
Goat complement.	0.0025	0.0015	0.0005	0.0002	0.0025	0.001	0.0005	0.0002	0.001	0.0005	0.0002	0.0001	0.005	0.0025	0.0005					0.05
Chicken complement.		0.07	0.01		0.01	0.005	0.001		0.05	0.03	0.002	0.001	0.07	0.03	0.005	0.002	0.03	0.015	0.0005	
Cat complement.	0.0025	0.0015	0.0007	0.0004	0.005	0.0015	0.0005	0.0002	0.025	0.015	0.0025	0.001	**0.004**	**0.001**	**0.0025**	**0.0005**				0.05
Dog complement.	0.005	0.0025	0.0008	0.0004	0.005	0.0025	0.0005	0.0002	**0.025**	**0.015**	**0.0025**	**0.001**		0.035	0.015	0.01				0.05

TABLE II.

Arrangement of different complements according to their capacity to reactivate different antihuman amboceptors.

Species of animal furnishing amboceptor.									
Rabbit	Amboceptor titer 0.0015 with guinea pig complement goat complement cat complement	Amboceptor titer 0.0025 with dog complement	Amboceptor titer 0.003 with rabbit complement	Amboceptor titer 0.005 with rat complement	Amboceptor titer 0.015 with pig complement	Amboceptor titer 0.025 with ox complement sheep complement	Amboceptor titer 0.07 with chicken complement		Amboceptor titer 0.025 with sheep complement
Guinea pig	Amboceptor titer 0.0005 with guinea pig complement	Amboceptor titer 0.0007 with rabbit complement	Amboceptor titer 0.001 with goat complement	Amboceptor titer 0.0015 with cat complement	Amboceptor titer 0.0025 with dog complement	Amboceptor titer 0.005 with chicken complement rat complement	Amboceptor titer 0.015 with pig complement	Amboceptor titer 0.02 with ox complement	
Dog	Amboceptor titer 0.0005 with cat complement	Amboceptor titer 0.001 with rabbit complement	Amboceptor titer 0.001 with pig complement	Amboceptor titer 0.015 with guinea pig complement dog complement cat complement	Amboceptor titer 0.02 with ox complement	Amboceptor titer 0.025 with chicken complement rat complement	Amboceptor titer 0.03 with chicken complement	Amboceptor had no action with sheep complement	
Cat	Amboceptor titer 0.001 with cat complement	Amboceptor titer 0.0025 with rabbit complement	Amboceptor titer 0.01 with goat complement	Amboceptor titer 0.015 with guinea pig complement pig complement	Amboceptor titer 0.03 with ox complement	Amboceptor titer 0.035 with chicken complement	Amboceptor titer 0.04 with dog complement rat complement	Amboceptor had no action with sheep complement	
Chicken	Amboceptor titer 0.015 with chicken complement	Amboceptor titer 0.03 with pig complement	Amboceptor had no action with rabbit, guinea pig, sheep, ox, rat, cat, goat, or dog complement.						

was employed in a dose of 0.1 cubic centimeter of a 10 per cent. suspension of washed human corpuscles. The total volume in each tube was made uniformly one cubic centimeter.

The above experiments revealed that a striking relation exists between the various complements and a given kind of amboceptor produced in different species of animals.

This is brought out in Table II.

With the amboceptors produced in guinea pig, cat, and chicken, the strongest action was obtained with their homologous complements. On the other hand, the amboceptors from the dog and rabbit acted more strongly with certain heterologous complements than with homologous complements. It was not the same heterologous complements, however, which showed this peculiarity. The strongest action with rabbit amboceptor was obtained with the complements of guinea pig, goat, cat, and dog. The complementary action of rabbit serum with dog amboceptor was, however, very much stronger than that of the dog complement with the amboceptor obtained from the dog. This dog amboceptor was thirty times more active in the presence of goat complement than in the presence of dog complement. Guinea pig complement, which has hitherto been considered universally suitable, is seen to be inferior to the complements of goat, rabbit, and pig when used with dog amboceptor, and it was without any complementary action for chicken amboceptor. The complements of ox and sheep, which were found to be quite suitable for goat amboceptor, are seen to be less suitable for the amboceptors derived from rabbit, guinea pig, dog, cat, and chicken. It is also remarkable that the sera of all mammals, except the pig, used in our experiments are devoid of any complementary action for chicken amboceptor, while chicken serum contains certain amounts of complement suitable for the amboceptors derived from those mammals.

Thus we have learned that the titers of an amboceptor may vary considerably according to the varieties of sera employed as complement. That the variations revealed in the titration of a given amboceptor are due to the variations in the complement content of the serum used as complement, can be further demonstrated by determining the limit of activity of the sera (serving as complement)

in the presence of a constant amount of amboceptor. In the following table is shown the comparative activity of different sera as complement. Rabbit amboceptor (in excess) was employed in this experiment.

TABLE III.

	Titration of sera as complement with antihuman rabbit amboceptor.									
	Guinea pig complement.	Cat complement.	Goat complement.	Dog complement.	Rabbit complement.	Rat complement.	Pig complement.	Ox complement.	Sheep complement.	Chicken complement.
Quantity for complete hemolysis	0.006	0.006	0.006	0.01	0.015	0.02	0.05	0.07	0.1	
Quantity for moderate hemolysis	0.003	0.003	0.003	0.005	0.007	0.01	0.03	0.035	0.05	0.07
Quantity for trace of hemolysis....................	0.002	0.002	0.002	0.003	0.004	0.005	0.015	0.015	0.02	0.02
Quantity for no hemolysis..	0.001	0.001	0.001	0.0015	0.002	0.003	0.007	0.008	0.01	0.01

Table III shows that the titration of the complementary activity of the sera of guinea pig, cat, and dog is about twice as strong as that of the rabbit for rabbit amboceptor. In this respect sheep serum was about sixteen times weaker, and chicken serum was too weak to produce complete hemolysis.

We have also studied the complementary activity of various sera for the amboceptors derived from heterologous as well as from homologous species. Our results are given in Table IV.

· This series of experiments demonstrates three interesting facts: (1) the preference of certain amboceptors for their homologous complements (chicken, guinea pig, goat); (2) the inferiority of certain complements for homologous amboceptors (rat); and (3) the increase of hemolytic activity of complements in general by the use of a larger amount of the amboceptor. The inferiority of homologous complements to certain heterologous complements has already been described with the sera derived from rabbits and dogs (see Table II). That cat amboceptor is more powerful with its homologous complement is shown in the same table. Thus, we find that the amboceptors derived from chicken, guinea pig, goat, and cat are stronger in the presence of their homologous complements,

TABLE IV.

Quantity of complement.	Rabbit complement.		Dog complement.		Cat complement.		Chicken complement.	Pig complement.	Pig complement.	Rat complement.	Guinea pig complement.		Guinea pig complement.		Goat complement.		Goat complement.	
	Rabbit amboceptor.		Dog amboceptor.		Cat amboceptor.		Chicken amboceptor.	Chicken amboceptor	Rat amboceptor.	Rat amboceptor.	Guinea pig ambocepto.		Goat amboceptor.		Guinea pig amboceptor.		Goat amboceptor.	
	(0.025)	(0.0025)	(0.02)	(0.0025)	(0.025)	(0.0025)	(0.1)	(0.05)	(0.1)	(0.1)	(0.025)	(0.002)	(0.09)	(0.005)	(0.025)	(0.0025)	(0.09)	(0.005)
0.1	C.H.	C.H.	Mch.H.	Mch.H.	C.H.	C.H.	C.H.	C.H.	C.H.	Sl.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.
0.05	C.H.	Md.H.	Tr.H.	Tr.H.	C.H.	C.H.	C.H.	Mch.H.	C.H.	No H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.
0.035	C.H.	Sl.H.	Tr.H.	No H.	C.H.	Sl.H.	Sl.H.	Tr.H.	C.H.		C.H.	C.H.	C.H.	Tr.H.	C.H.	C.H.	C.H.	C.H.
0.02	C.H.	Tr.H.	No H.		C.H.	No H.	No H.	No H.	Mch.H.		C.H.	No H.	C.H.	No H.	C.H.	Md.H.	C.H.	C.H.
0.01	C.H.	Tr.H.			Mch.H.				Tr.H.		C.H.		Mch.H.		Mch.H.	No H.	C.H.	Sl.H.
0.005	C.H.	No H.			Tr.H.				No H.		Mch.H.		Sl.H.		No H.		Md.H.	No H.
0.002	Md.H.				No H.						No H.		Tr.H.				Tr.H.	
0.001	No H.												No H.				No H.	

C.H. = complete hemolysis; Mch.H. = much hemolysis; Md.H. = moderate hemolysis; Sl.H. = slight hemolysis; Tr.H. = trace of hemolysis.

while those from rabbit, dog, and rat are stronger in the presence of certain suitable heterologous complements.

After studying the complex inter-relation of various complements and amboceptors, we took up the further study of a few complements. The sera of pig, sheep, and ox were studied especially because we hoped to be able to utilize them as complement, for they may be obtained in large quantity from any abattoir. We soon discovered, however, that they were quite unsuitable for the syphilis reaction. In the first place, ox serum often contains a variable and sometimes considerable amount of natural amboceptor for human erythrocytes, and for this reason we did not pursue the study of this serum.

The sera of pig and sheep are usually devoid of the natural anti-human amboceptor. They were examined for their complementary activity for rabbit amboceptor. The results are shown in Tables V and VI.

The striking feature of the sera of these two animals is the rapid disappearance of their complementary activity. It is more marked with pig serum. Both varieties of serum became so weak after forty-eight hours (0° C.) that they no longer produced complete hemolysis in a quantity as large as 0.1 cubic centimeter. Guinea pig serum, under the same conditions, showed practically no diminution during the same period of preservation. The rapid deterioration of both pig and sheep serum renders them unreliable because of the constantly decreasing activity of complement. The rate of deterioration is also quite irregular with different samples of the same variety of serum. Thus, in point of reliability and activity, they are far inferior to the serum of the guinea pig and are not recommended for use in the syphilis reaction.

CONCERNING THE USE OF VARIOUS SERA AS COMPLEMENT IN THE
SERUM REACTION OF SYPHILIS.

In deciding whether or not the serum of a given animal is suitable as complement in the complement deviation test, we have to consider two essential properties of the serum. The first is, of course, its complementary activity, and the second is its susceptibility to fixation. We have already studied in detail the comparative com-

TABLE V.

Pig Serum.

Serial number of pigs.	Titration with antihuman rabbit amboceptor, o.o1 c.c.									Titrated for natural anti-human amboceptor.	
	4 hours old.			24 hours old.			48 hours old.			4 hours old.	
										Active. 0.2	Inactivated at 56°C. 0.2 +0.05 guinea pig serum.
	0.1	0.05	0.025	0.1	0.05	0.025	0.1	0.05	0.025		
No. 1	C.H.	C.H.	C.H.	C.H.	Mch.H.	Sl.H.	Sl.H.	Tr.H.	No H.	No H.	No H.
No. 2	C.H.	C.H.	C.H.	C.H.	C.H.	Mch.H.	Sl.H.	Tr.H.	No H.	No H.	No H.
No. 3	C.H.	C.H.	C.H.	C.H.	C.H.	Mch.H.	Md.H.	Tr.H.	No H.	No H.	No H.
No. 4	C.H.	C.H.	C.H.	C.H.	Mch.H.	Sl.H.	Sl.H.	No H.	No H.	No H.	No H.
No. 5	C.H.	C.H.	C.H.	C.H.	C.H.	Mch.H.	Mch.H.	Tr.H.	No H.	No H.	No H.
No. 6	C.H.	C.H.	C.H.	C.H.	C.H.	Sl.H.	Mch.H.	Tr.H.	No H.	No H.	No H.
No. 7	C.H.	C.H.	C.H.	C.H.	Mch.H.	Mch.H.	Sl.H.	No H.	No H.	No H.	No H.
No. 8	C.H.	C.H.	C.H.	Mch.H.	Mch.H.	Sl.H.	Sl.H.	No H.	No H.	No H.	No H.
No. 9	C.H.	C.H.	C.H.	C.H.	Sl.H.	Tr.H.	Sl.H.	No H.	No H.	No H.	No H.
No. 10	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	No H.	No H.
Guinea pig serum. (control)	C.H.	C.H.	C.H.								

TABLE VI.

Sheep Serum.

Serial number of sheep.	Titration with antihuman rabbit amboceptor, 0.01 c.c.									Titrated for natural antihuman amboceptor.	
	4 hours old.			24 hours old.			48 hours old.			4 hours old. 0.2	Inactivated at 56°C. 0.2
	0.1	0.05	0.025	0.1	0.05	0.025	0.1	0.05	0.025	Active.	+ 0.05 guinea pig serum.
No. 1	C.H.	Almost C.H.	Mch.H.	C.H.	Mch.H.	No H.	Mch.H.	Tr.H.	No H.	No H.	Tr.H.
No. 2	C.H.	Almost C.H.	Mch.H.	Almost C.H.	Tr.H.	No H.	Sl.H.	No H.	No H.	No H.	No H.
No. 3	C.H.	Almost C.H.	Mch.H.	C.H.	Sl.H.	No H.	Almost C.H.	No H.	No H.	No H.	No H.
No. 4	C.H.	C.H.	Mch.H.	C.H.	Almost C.H.	Sl.H.	Mch.H.	Sl.H.	Tr.H.	Tr.H.	Sl.H.
No. 5	C.H.	C.H.	Mch.H.	C.H.	C.H.	No H.	C.H.	Tr.H.	No H.	No H.	No H.
No. 6	C.H.	C.H.	Mch.H.	C.H.	Mch.H.	Tr.H.	Sl.H.	No H.	No H.	No H.	No H.
No. 7	C.H.	C.H.	Mch.H.	C.H.	Almost C.H.	No H.	Md.H.	Tr.H.	No H.	No H.	Tr.H.
No. 8	C.H.	C.H.	Mch.H.	C.H.	C.H.	Tr.H.	C.H.	Almost C.H.	Tr.H.	Tr.H.	Sl.H.
Guinea pig serum (control)	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.		

plementary activities of the sera of various species of animals. We propose to report here, therefore, the results which we have obtained with regard to their comparative fixability.

As mentioned at the beginning of this article, Noguchi long ago showed that the complement of goat serum remains undeviated by specific precipitates or by the syphilis reaction. This phenomenon was decidedly more pronounced when the goat furnished both complement and amboceptor. He has also demonstrated that rabbit serum is less sensitive to fixation than guinea pig serum. Hence he suspected that the amboceptor might have such a strong affinity toward its homologous complement as to reverse partially the process of fixation. The question of whether or not there is such a relation between the source of amboceptor and the fixability of complements was left unsettled. This was one of the problems which we set out to solve, and our results will now be given.

The fixation test was carried out with Noguchi's system in the usual way. The positive serum was from a case of untreated secondary syphilis, and, in the quantity of 0.002 cubic centimeter, was capable of fixing completely 0.04 cubic centimeter of guinea pig serum. In the following experiments, 0.02 cubic centimeter of the syphilitic serum was used for each tube.

TABLE VII.

	COMPLEMENT.				
	Guinea pig 0.04 (2 units).	Rabbit No. 1 0.08 (2 units).	Rabbit No. 2 0.08 (2 units).	Goat No. 1 0.04 (2 units).	Goat No. 2 0.04 (2 units).
Guinea pig amboceptor (2 units)	No hemolysis	Partial hemolysis (no hemolysis for some time).	Partial hemolysis (no hemolysis for some time).	Complete hemolysis (much delay).	Complete hemolysis (much delay).
Rabbit amboceptor (2 units).	No hemolysis	Partial hemolysis (no hemolysis for some time).	Partial hemolysis (no hemolysis for some time).	Complete hemolysis (much delay).	Complete hemolysis (much delay).
Goat amboceptor (2 units).	Slight hemolysis (no hemolysis for some hours).	Much hemolysis (no hemolysis for some time).	Much hemolysis (no hemolysis for some time).	Complete hemolysis (slight delay).	Complete hemolysis (slight delay).

The experiments given above show that the complement contained in goat serum is totally unfixable. There was some delay in commencing hemolysis, but it was finally completed within a few hours. The complement contained in rabbit serum showed the peculiarity that hemolysis commenced after six hours or so, and after about twenty-four hours a moderate amount of hemolysis was observed. This is most striking when compared with the complete and lasting inhibition of hemolysis in the case of guinea pig complement. The use of goat amboceptor, however, has caused more or less hemolysis even with guinea pig complement. Experiments also show that when the complements are fixable the homologous amboceptor and complements do not influence the fixation phenomenon. It is difficult to explain why the fixation of guinea pig complement is imperfect when tested by goat amboceptor. It may be due to the presence in guinea pig serum of a non-fixable complement, which, while quite active with the goat amboceptor, is inactive with the amboceptors from rabbit and guinea pig. Now, with regard to the state of non-fixation of the complement of goat serum, there are two possibilities. The first is its insensitiveness to fixation. The delay in commencing hemolysis may be accounted for by assuming that the quantity of complement still free in the mixture was considerably reduced by a partial fixation. The second is the gradual liberation of complement already fixed through the introduction of the goat amboceptor. Apart from these hypothetical considerations, the above experiments showed that rabbit and goat complement and goat amboceptor are unsuitable in deviation tests.

We have determined the fixability of the complements contained in the sera of pigs and sheep. Pig complement was fixable, while that of sheep was somewhat less so. But, as has already been pointed out, their complementary activity is so feeble that, in order to employ two units, their absolute quantities may reach 0.1 cubic centimeter or even exceed this. If the fixation were *selectively* directed to the complement, an accurate result could always be secured by employing any quantity in which two activity-units existed. But as we shall describe elsewhere, the fixation is not selectively directed to the complement. The presence of non-complementary protein constituents may, therefore, interfere with the

fixation of complement. From this it will be easy to understand why we must avoid weak sera, for in these the quantity which contains a single unit of complement is too large.

CONCLUSIONS.

1. The maximum activity of an antihuman hemolytic amboceptor may be obtained by employing the homologous or heterologous complement, according to the variable relations existing between the species furnishing the amboceptor and the one supplying the complement. Thus, some amboceptors are best reactivated by the complement of the same species, while others may act most strongly when reactivated with the complements of certain suitable heterologous species.

2. From the above it is clear that the complementary activity of a given serum may be very variable according to the varieties of amboceptors employed. In expressing the complementary activity of a serum, the species of the host of the amboceptor must always be stated. Thus, one serum may have many different complementary titers according to the amboceptors used. A similar variation in the titers of the amboceptors occurs when a variety of complements are employed.

3. Certain species of animals (pig and sheep) yield sera which are comparatively poor in reactivating most varieties of antihuman amboceptors. The complements of these species deteriorate rapidly.

4. The serum of chicken contained but little complement for the amboceptors derived from the mammalia, while the amboceptor from the chicken was only poorly, or not at all, reactivable by the complements contained in the mammalian sera. The serum of pig was the only variety which reactivated this amboceptor in a fair degree.

5. For the fixation tests guinea pig complement is the most favorable. This complement is also the most active and durable of those which have been studied. The complements of pig and sheep are quite fixable, but their weakness and rapid deterioration render them unsuitable for fixation purposes. Rabbit complement is quite active but is not easily fixable. Goat complement is, as already

stated, difficult to fix, in spite of its strong complementary activity. The other complements are unsuitable because of their feeble complementary activity.

6. For fixation tests the antihuman amboceptors produced in the rabbit and guinea pig are suitable. They are, moreover, very active and do not cause the phenomenon of non-fixation. The amboceptors from other animals are unsuitable, as we cannot find a complement which strongly reactivates them. The amboceptor from the goat is unsuitable because of the danger of masking the fixation phenomenon by subsequent hemolysis.

7. In summing up, we arrive at the conclusion that the rabbit is the best animal for producing antihuman amboceptor, and the guinea pig for supplying complement. The guinea pig produces a good amboceptor, but its small size renders it second in choice.

So far, no other animals have been found useful for the fixation experiments.

THE INTERFERENCE OF INACTIVE SERUM AND EGG-WHITE IN THE PHENOMENON OF COMPLEMENT FIXATION

By HIDEYO NOGUCHI and J. BRONFENBRENNER

THE INTERFERENCE OF INACTIVE SERUM AND EGG-WHITE IN THE PHENOMENON OF COMPLEMENT FIXATION.[1]

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Is the fixative action of a specific antigen-antibody combination directed only towards the serum constituents known as complement or towards any protein whose physico-chemical properties with regard to the fixation phenomenon are similar to complement? This question needs experimental determination, as the complementary activity of a given specimen of serum has no constant quantitative relationship to the amount of other non-complementary protein constituents contained in the same serum.

If the fixation were directed exclusively towards the active complementary constituents, we could measure quantitatively the fixing capacity of the combination of a given specific antigen and antibody by simply estimating the units of complement fixed. On the other hand, if the fixation is also directed towards the other indifferent proteins simultaneously present in the complement-containing serum, the quantitative estimation of the fixative capacity of the antigen-antibody combination becomes complicated; we cannot measure it by the units of complement fixed, for the fixation is also shared by the other serum components whose exact amounts cannot be ascertained by this method. A great miscalculation of the real fixing capacity must inevitably result when one uses as indicator the complementary activity alone. This source of error is steadily increased by the gradual disappearance of the complementary activity from the serum, while the amount of the other non-complementary constituents not only remains undiminished, but is increased by the gradual conversion of the active complement into an

[1] Received for publication, September 21, 1910.

inactive complementoid. Before this fundamental relation is established, any attempt to estimate complement fixation quantitatively is premature.

In this communication we present the results which we obtained in studying the effects of various non-complementary proteins upon the complement fixation phenomenon, including the syphilis reaction. In our study we employed, as usual, the antihuman hemolytic system recommended by Noguchi. Every factor concerned in this system is under perfect quantitative control.

THE SYPHILIS REACTION.

In this series the effects of various inactivated or spontaneously deteriorated sera and egg-white were studied. Varying quantities of each serum and of egg-white, in a twenty per cent. dilution in 0.9 per cent. salt solution, were added under three different conditions to the fixing mixture of syphilitic serum and antigen (aceton-insoluble fraction of lipoid from human liver). They were added and incubated (1) before the addition of complement; (2) at the same time as the complement; and (3) after the complement had been fixed. Some tubes without any serum or egg-white were, of course, provided as controls. The amount of syphilitic serum used was 0.02 cubic centimeter for each tube, and this amount was capable of fixing 0.08 cubic centimeter of guinea pig complement in the presence of 0.1 cubic centimeter (four units) of the antigen emulsion. In the case of non-anticomplementary sera, the amount of complement used was uniformly 0.04 cubic centimeter of fresh guinea pig serum. On the other hand, certain deteriorated sera showed a more or less anticomplementary property. This had to be overcome by adding, in addition to the 0.04 cubic centimeter of complement, the exact amount of active complement (guinea pig serum) necessary to neutralize each such specimen. The total volume in each tube was made equal to one cubic centimeter. The incubation period was one hour at 37° C. The first and third series in Table I were twice incubated, as this was required by the nature of the experiments. Then 0.1 cubic centimeter of a 10 per cent. suspension of washed human corpuscles and two units of anti-

TABLE I.

	1st series. Inactive serum added before complement.			2d series. Inactive serum and complement introduced at same time.			3rd series. Complement added before inactive serum.		
	0.1	0.05	0.03	0.1	0.05	0.03	0.1	0.05	0.03
Guinea pig serum No. 1, inactivated by age...	C.H.	Mch.H.	Sl.H.	Mch.H.	Md.H.	Tr.H.	No H.	No H.	No H.
Guinea pig serum No. 2, inactivated by age...	C.H.	Mch.H.	Sl.H.	Mch.H.	Md.H.	Tr.H.	No H.	No H.	No H.
Guinea pig serum No. 3, inactivated, 56°C...	C.H.	Md.H.	Sl.H.	Mch.H.	Md.H.	Tr.H.	No H.	No H.	No H.
Guinea pig serum No. 4, inactivated, 56°C...	C.H.	Md.H.	Sl.H.	Mch.H.	Md.H.	Tr.H.	No H.	No H.	No H.
Human serum No. 1, inactivated by age...	Sl.H.	No H.	No H.	Tr.H.	No H,	No H.	No H.	No H.	No H.
Human serum No. 2, inactivated by age...	Mch.H.	Sl.H.	No H.	Sl.H.	No H,	No H.	No H.	No H.	No H.
Human serum No. 3, inactivated by age...	No H.	No H.	No H.	No H.	No H,	No H.	No H.	No H.	No H.
Human serum No. 4, inactivated, 56°C...	C.H.	Mch.H.	Sl.H.	Md.H.	Sl.H.	No H.	No H.	No H.	No H.
Human serum No. 5, inactivated, 56°C...	Mch.H.	Sl.H.	No H.	Sl.H.	Sl.H.	No H.	No H.	No H.	No H.
Sheep serum No. 1, inactivated by age...	C.H.			Mch.H.		No H.	No H.		
Sheep serum No. 2, inactivated 56°C...	C.H.			Mch.H.		No H.	No H.		
Pig serum, inactivated by age...	C.H.			Mch.H.		No H.	No H.		
Horse serum, inactivated, 56°C...	C.H.			Mch.H.		No H.	No H.		
Cat serum, inactivated, 56°C...	C.H.			Mch.H.		No H.	No H.		
Dog serum, inactivated, 56°C...	Mch.H.	Sl.H.	No H.	Sl.H.	No H.	No H.	No H.		
Goat serum No. 1, inactivated by age...	Sl.H.	No H.	No H.	No.H.	No H.	No H.	No H.		
Goat serum No. 2, inactivated, 56°C...	Mch.H.	Sl.H.	No H.	Mch.H.	Sl.H.	No H.	No H.		
Chicken serum, inactivated by age...	C.H.	Mch.H.	Sl.H.	Md.H.	Sl.H.	No H.	No H.		
Chicken serum, inactivated, 56°C...	C.H.	Mch.H.	No H.	Mch.H.	Tr.H.	Sl.H.	No H.		
Egg-white (20%), unheated...	Almost C.H.	Almost C.H.	Md.H.	Almost C.H.	Mch.H.	Sl.H.	No H.	No H.	No H.
Egg-white (20%), heated to 56°C...	C.H.	Mch.H.	Sl.H.	Mch.H.	Sl.H.	No H.	No H.	No H.	No H.

human amboceptor (rabbit) were added to all tubes, mixed well, and incubated for two hours at 37° C. The results are given in Table I.

The foregoing experiments demonstrate several important points. The first series shows that the addition of various animal sera, from which the complementary property had disappeared either by a spontaneous deterioration or by an artificial inactivation, to the fixing mixture of syphilitic serum and antigen, saturated completely the latter's fixing capacity so that it was no longer capable of fixing the complement of fresh guinea pig serum subsequently introduced. Exceptions were encountered with human and goat sera, which behaved inconstantly. While some of the human sera interfered with the fixation to the same degree as that of most animal sera, others showed but little interference. The serum of the goat was also comparatively less interfering. The most remarkable fact in the series is that the egg-white acted in the same way as most animal sera.

In the second series we observe somewhat less interference of fixation by the sera and egg-white. This is not at all surprising, because of their simultaneous addition with the complement, under which condition the fixing had been directed to both. In the third series there was no hemolysis. It is evident that the subsequent addition of these substances had no reversing effect upon the complement, which had been fixed before they were introduced.

.The deductions which may be drawn from these observations are that the fixing capacity of the syphilitic serum and antigen can be saturated not only by active complement-containing sera or inactive sera, but also by an apparently indifferent suspension of proteins (egg-white), so long as their physico-chemical properties are the same. It also shows how erroneous it is to estimate the fixing capacity by the estimation of one biological property (complementary) without reference to the ratio of this biological activity to the other components of the serum serving as complement. For quantitative work with the complement fixation test, the establishment of a standard ratio between the complementary unit and the volume of the serum, becomes essential. In addition to this,

the relation of the fixability of the sera of different species must be considered.

. *The Complement Fixation by Specific Precipitate* (*Bordet-Gengou Phenomenon*).—Parallel series of experiments with the foregoing were also made with the precipitate produced by the anti-meningococcic horse serum (Flexner and Jobling) and meningococcic extract.

The results obtained were in perfect harmony with those of the syphilis reaction just described; hence protocols are not called for.

The fixability of these sera and egg-white diminished progressively at temperatures above 56° C., and disappeared at 85° C.

Alcohol, which coagulates proteins, removes the interfering property of sera and egg-white.

CONCLUSIONS.

The fixing property of a specific precipitate and of syphilitic serum in the presence of certain antigenic lipoids, can be removed by adding certain non-complementary proteins of blood serum or hen's egg.

This disappearance of the complementary activity in the syphilis reaction, as well as in the true Bordet-Gengou reaction, is a phenomenon which incidentally accompanies the fixation of certain serum constituents, some of which possess a complementary activity. The presence or absence of the complementary property in these protein components does not influence fixation. Whether the disappearance of the complementary activity during the phenomenon of so-called fixation is due to a mechanical precipitation of the molecules through absorption or whether it is due to a physico-chemical alteration of the active molecules, is unknown. It is more probable that a chemical interaction takes place in the case of the syphilis reaction. Certain sera, for example, those derived from man and goat, show a low fixability.

It is interesting to note that the fixability is gradually diminished when these sera and egg-white are heated to a temperature above 56° C., and totally disappears at 85° C. The coagulation of proteins with absolute alcohol or by boiling, destroys their interfering property.

The fact that the fixation is not selectively directed towards complement, has a very important meaning for exact serology. The one-sided accuracy as to the complementary unity is no longer sufficient for quantitative work. Both the complementary and the volumetric unity of a serum serving as the source of complement should be taken into consideration. Besides, the fixability of the sera of various species of animals must also be considered.

From these facts a formula may be derived for deciding the degree of suitableness of a serum.

$$X = K \cdot \frac{P}{V}$$

X is the degree of suitableness; K, the species constant for the fixability; P, the complementary activity; and V, the volume of serum. It will be seen that the suitableness is proportional to the fixability constant and the complementary unity, and inversely proportional to the volume of serum employed.

As to what species yields the largest value for X, we refer the reader to our studies published elsewhere.[2]

[2] (1) Variations in the Complement Activity and Fixability of Guinea pig Serum; (2) Comparative Merits of Various Complements and Amboceptors in the Serum Diagnosis of Syphilis, this number of the *Journal of Experimental Medicine.*

THE INTERFERENCE OF INACTIVE SERUM AND EGG-WHITE IN THE PHENOMENON OF COMPLEMENT FIXATION

By HIDEYO NOGUCHI AND J. BRONFENBRENNER

THE INTERFERENCE OF INACTIVE SERUM AND EGG-WHITE IN THE PHENOMENON OF COMPLEMENT FIXATION.[1]

BY HIDEYO NOGUCHI AND J. BRONFENBRENNER.

(*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*)

Is the fixative action of a specific antigen-antibody combination directed only towards the serum constituents known as complement or towards any protein whose physico-chemical properties with regard to the fixation phenomenon are similar to complement? This question needs experimental determination, as the complementary activity of a given specimen of serum has no constant quantitative relationship to the amount of other non-complementary protein constituents contained in the same serum.

If the fixation were directed exclusively towards the active complementary constituents, we could measure quantitatively the fixing capacity of the combination of a given specific antigen and antibody by simply estimating the units of complement fixed. On the other hand, if the fixation is also directed towards the other indifferent proteins simultaneously present in the complement-containing serum, the quantitative estimation of the fixative capacity of the antigen-antibody combination becomes complicated; we cannot measure it by the units of complement fixed, for the fixation is also shared by the other serum components whose exact amounts cannot be ascertained by this method. A great miscalculation of the real fixing capacity must inevitably result when one uses as indicator the complementary activity alone. This source of error is steadily increased by the gradual disappearance of the complementary activity from the serum, while the amount of the other non-complementary constituents not only remains undiminished, but is increased by the gradual conversion of the active complement into an

[1] Received for publication, September 21, 1910.

inactive complementoid. Before this fundamental relation is established, any attempt to estimate complement fixation quantitatively is premature.

In this communication we present the results which we obtained in studying the effects of various non-complementary proteins upon the complement fixation phenomenon, including the syphilis reaction. In our study we employed, as usual, the antihuman hemolytic system recommended by Noguchi. Every factor concerned in this system is under perfect quantitative control.

THE SYPHILIS REACTION.

In this series the effects of various inactivated or spontaneously deteriorated sera and egg-white were studied. Varying quantities of each serum and of egg-white, in a twenty per cent. dilution in 0.9 per cent. salt solution, were added under three different conditions to the fixing mixture of syphilitic serum and antigen (aceton-insoluble fraction of lipoid from human liver). They were added and incubated (1) before the addition of complement; (2) at the same time as the complement; and (3) after the complement had been fixed. Some tubes without any serum or egg-white were, of course, provided as controls. The amount of syphilitic serum used was 0.02 cubic centimeter for each tube, and this amount was capable of fixing 0.08 cubic centimeter of guinea pig complement in the presence of 0.1 cubic centimeter (four units) of the antigen emulsion. In the case of non-anticomplementary sera, the amount of complement used was uniformly 0.04 cubic centimeter of fresh guinea pig serum. On the other hand, certain deteriorated sera showed a more or less anticomplementary property. This had to be overcome by adding, in addition to the 0.04 cubic centimeter of complement, the exact amount of active complement (guinea pig serum) necessary to neutralize each such specimen. The total volume in each tube was made equal to one cubic centimeter. The incubation period was one hour at 37° C. The first and third series in Table I were twice incubated, as this was required by the nature of the experiments. Then 0.1 cubic centimeter of a 10 per cent. suspension of washed human corpuscles and two units of anti-

TABLE I.

	1st series. Inactive serum added before complement.			2d series. Inactive serum and complement introduced at same time.			3rd series. Complement added before inactive serum.		
	0.1	0.05	0.03	0.1	0.05	0.03	0.1	0.05	0.03
Guinea pig serum No. 1, inactivated by age.	C.H.	Mch.H.	Sl.H.	Mch.H.	Md.H.	Tr.H.	No H.	No H.	No H.
Guinea pig serum No. 2, inactivated by age.	C.H.	Mch.H.	Sl.H.	Mch.H.	Md.H.	Tr.H.	No H.	No H.	No H.
Guinea pig serum No. 3, inactivated, 56°C.	C.H.	Md.H.	Sl.H.	Mch.H.	Md.H.	Tr.H.	No H.	No H.	No H.
Guinea pig serum No. 4, inactivated, 56°C.	C.H.	Md.H.	Sl.H.	Mch.H.	Md.H.	Tr.H.	No H.	No H.	No H.
Human serum No. 1, inactivated by age.	Sl.H.	No H.	No H.	Tr.H.	No H,	No H.	No H.	No H.	No H.
Human serum No. 2, inactivated by age.	Mch.H.	Sl.H.	No H.	Sl.H.	No H.	No H.	No H.	No H.	No H.
Human serum No. 3, inactivated, 56°C.	No H.	No H.	Sl.H.	No H.	No H.	No H.	No H.	No H.	No H.
Human serum No. 4, inactivated, 56°C.	C.H.	Mch.H.	No H.	Mch.H.	Sl.H.	No H.	No H.	No H.	No H.
Human serum No. 5, inactivated, 56°C.	Mch.H.	Sl.H.	No H.	Md.H.	Sl.H.	No H.	No H.	No H.	No H.
Sheep serum No. 1, inactivated by age.	C.H.			Mch.H.			No H.		
Sheep serum No. 2, inactivated 56°C.	C.H.			Mch.H.			No H.		
Pig serum, inactivated, 56°C.	C.H.			Mch.H.			No H.		
Horse serum, inactivated, 56°C.	C.H.			Mch.H.			No H.		
Cat serum, inactivated, 56°C.	C.H.			Mch.H.			No H.		
Dog serum, inactivated, 56°C.	Mch.H.	Sl.H.	No H.	Sl.H.	No H.	No H.	No H.		
Goat serum No. 1, inactivated by age.	Sl.H.	No H.	No H.	No.H.	No H.	No H.	No H.		
Goat serum No. 2, inactivated, 56°C.	Mch.H.	Sl.H.	Sl.H.	Md.H.	Sl.H.	No H.	No H.		
Chicken serum, inactivated by age.	C.H.	Mch.H.	No H.	Mch.H.	Sl.H.	No H.	No H.		
Chicken serum, inactivated, 56°C.	C.H.	Mch.H.	Md.H.	Md.H.	Tr.H.	No H.	No H.		
Egg-white (20%), unheated.	C.H.	Almost C.H.	Md.H.	Almost C.H.	Mch.H.	Sl.H.		No H.	No H.
Egg-white (20%), heated to 56°C.	C.H.	Mch.H.	Sl.H.	Mch.H.	Sl.H.	No H.		No H.	No H.

human amboceptor (rabbit) were added to all tubes, mixed well, and incubated for two hours at 37° C. The results are given in Table I.

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CHANGES IN THE PROTEOLYTIC ENZYMES AND ANTI-ENZYMES OF THE BLOOD SERUM PRODUCED BY SUBSTANCES (CHLOROFORM AND PHOSPHORUS) WHICH CAUSE DEGENERATIVE CHANGES IN THE LIVER

By EUGENE L. OPIE, BERTHA I. BARKER AND A. R. DOCHEZ

CHANGES IN THE PROTEOLYTIC ENZYMES AND ANTI-ENZYMES OF THE BLOOD SERUM PRODUCED BY SUBSTANCES (CHLOROFORM AND PHOSPHORUS) WHICH CAUSE DEGENERATIVE CHANGES IN THE LIVER.[1]

BY EUGENE L. OPIE, BERTHA I. BARKER AND A. R. DOCHEZ.

(From the Laboratories of the Rockefeller Institute for Medical Research, New York.)

The observations of Salkowski,[2] which have shown that incubated liver undergoes "self-digestion," have been extended to almost all tissues of the animal body. It is well known that the autolysis of most tissues occurs with maximum activity in a weakly acid medium. Biondi[3] has found that disintegration of liver is hastened by addition of weak acid, and Hedin and Rowland,[4] using the expressed juice of various organs, have noted that spleen, kidney, and lymphatic nodes autolyze more actively in acid than in neutral or alkaline media. The trivial autolysis of voluntary muscle is not materially affected by acid or alkali, but the autolysis of heart muscle resembles that of other organs. Levene and Stookey[5] have found that autolysis of brain tissue and of testicle is favored by the presence of acid.

The pancreas is not an exception to the foregoing rule. The well-known observations of Heidenhain[6] have shown that trypsin, which exhibits maximum activity in alkali, exists in the pancreas as inactive zymogen, so that fresh extracts of the organ cause little proteolysis under conditions which favor the activity of trypsin.

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[1] Received for publication, November 4, 1910.
[2] Salkowski, *Ztschr. f. klin. Med.*, 1890, xvii, Suppl., p. 77.
[3] Biondi, *Virchows Arch. f. path. Anat.*, 1896, cxliv, 373.
[4] Hedin and Rowland, *Ztschr. f. physiol. Chem.*, 1901, xxxii, 531.
[5] Levene and Stookey, *Jour. Med. Research*, 1903, x, 212.
[6] Heidenhain, *Arch. f. d. ges. Physiol.*, 1875, x, 557.

The conditions under which acid activates pancreatic extracts and their relation to the activation of pancreatic juice by enterokinase have been studied by one of us (Dochez).[7] Fresh pancreatic tissue which undergoes active autolysis in the presence of weak acid, suffers little change in neutral solutions and is almost unchanged after incubation in an alkaline medium, the concentration of which agrees with that favorable to the action of trypsin (0.2 to 0.4 per cent. sodium carbonate). Heidenhain has shown that acid converts tryptic zymogen of fresh pancreas into trypsin; pancreas subjected to the action of acid acquires the power to digest under conditions which are favorable to trypsin, namely, in neutral or alkaline media. Although splenic substance autolyzes with greater activity in acid than in alkali, Hedin[8] has obtained in relatively purified form two enzymes, one of which, designated α-lieno-protease, acts in alkali, whereas the other, designated β-lieno-protease, acts in acid. If fresh spleen (Hedin[9]) is temporarily subjected to the action of weak acid, its ability to autolyze in the presence of alkali is materially increased. Pretreatment with acid makes active an enzyme which digests in the presence of alkali.

Dochez[10] has studied the modifications of hepatic autolysis which occur as the result of changes in reaction, and he has compared autolysis of liver with autolysis of fresh pancreas. Whereas fresh liver autolyzes with greater activity in acid (0.2 per cent. acetic acid) than in neutral or alkaline media (from 0.2 to 0.4 per cent. sodium carbonate), liver which has been treated with weak acid during twenty-four hours acquires the power to autolyze or to digest casein in alkaline medium with equal or greater activity than in acid. Other evidence cited by Dochez shows that the liver contains two enzymes, one of which acts in acid, whereas the other, which acts in alkali, is active only after the tissue has been subjected to acid. Vernon[11] has shown that pancreatic extracts become active after standing; Dochez has likewise noted that liver tissue allowed to stand on ice, gradually acquires the power to autolyze in the presence of alkali.

[7] Dochez, *Proc. Soc. Exper. Biol. and Med.*, 1910, vii, 97.

[8] Hedin, *Jour. Physiol.*, 1904, xxx, 155.

[9] Hedin, Festschrift für Olof Hammarsten, Upsala, 1906, vi, 1.

[10] Dochez, *Jour. Exper. Med.*, 1910, xii, 666.

[11] Vernon, *Jour. Physiol.*, 1901, xxvii, 269.

It is noteworthy that the polynuclear leucocytes of an inflammatory exudate cause proteolysis with greater activity in alkali than in acid and, unlike pancreas, liver or spleen and, doubtless, other organs, exhibit this activity in alkali when freshly obtained from the body; no pretreatment with acid is necessary in order to bring this enzyme (leucoprotease) into action. The bone marrow, unlike other tissues, exhibits the same ability to digest with greater activity in alkali than in acid.

The power of the blood serum to restrain the activity of tryptic digestion has long been known. Hahn[12] made the observation that trypsin fails to digest fibrin or gelatin when fresh blood serum is present. He found that this power to inhibit is lost if blood serum is heated to a temperature of 65° C. The inhibiting substance is apparently attached to the proteins of the serum, but is not a common property of all proteins of the blood, for the globulin fraction of the serum, precipitated by half saturation with ammonium sulphate, fails to restrain the action of trypsin, whereas the albumin fraction precipitated by complete saturation after globulin has been removed exerts a retarding influence.

Ascoli and Bezzola,[13] in 1903, doubtless influenced by the observations of Fr. Müller[14] upon self-digestion of lung consolidated by pneumonia, have sought to determine if this inhibiting property of the serum undergoes any alteration during the progress of pneumonia. They have claimed that there is at first a marked increase of anti-tryptic action; this increase is maintained for a time, and after crisis it is followed by a decrease, often occurring in association with disappearance of the local lesion. The subject attracted little attention until Brieger and Trebing[15] showed that there is an almost constant increase of the anti-tryptic activity of the blood serum in association with carcinoma. Subsequent studies have demonstrated that this increased anti-enzymotic activity is apparently dependent upon the accompanying cachexia rather than upon the new growth itself, and a similar change has been observed in association with a variety of diseases, such as pneumonia, typhoid

[12] Hahn, *Berl. klin. Wchnschr.,* 1897, xxxiv, 499.

[13] Ascoli and Bezzola, *Berl. klin. Wchnschr.,* 1903, xl, 391.

[14] Fr. Müller, *Verhandl. d. 20 Cong. f. inn. Med.,* 1902, 192.

[15] Brieger and Trebing, *Berl. klin. Wchnschr.,* 1908, lxv, 1041.

fever, tuberculosis, and exophthalmic goitre, of which the common characteristic, according to K. Meyer,[16] is increased disintegration of the protein constituents of the body, perhaps referable to increased activity of proteolytic enzymes.

Finding that the serum in certain diseases associated with leucocytosis exhibits increased anti-enzymotic activity when tested with enzyme of polynuclear leucocytes, several observers (Bittorf,[17] Wiens[18]) have maintained that the enzyme set free by leucocytes neutralizes the anti-body of the serum and thus indirectly causes an excessive regeneration. Nevertheless, numerous observations have shown, on the one hand, that anti-enzymotic activity of the blood serum is not constantly increased in association with leucocytosis (K. Meyer), whereas, on the other hand, many conditions, such as malignant growth, tuberculosis, and typhoid fever, frequently accompanied by increase of anti-enzyme, usually exhibit no leucocytosis.

By a series of comparative tests, Jochmann and Kantorowicz[19] have shown that serum which exhibits high anti-enzymotic activity toward trypsin shows increased ability to restrain the activity of the enzyme of the polynuclear leucocytes, which like trypsin digests in the presence of an alkaline medium. Injection of trypsin into rabbits increases the ability of the serum to inhibit both trypsin and leucoprotease, and, furthermore, injection of leucoprotease has the same result.

The occurrence of increased anti-enzyme in the serum suggests the possibility that anti-enzyme is increased in order to balance an increased quantity of free enzyme. With this possibility in view, we have attempted to determine if the proteolytic activity of the blood serum undergoes any alteration during the course of chloroform poisoning so severe that advanced degenerative changes are produced in the liver. The possibility that such a study might prove fruitful has been suggested by the observation that increase of anti-leucoprotease accompanies prolonged intoxication with chloroform or phosphorus.

[16] K. Meyer, *Berl. klin. Wchnschr.*, 1909, xlvi, 1064, 1890.

[17] Bittorf, *Deutsch. Arch. f. klin. Med.*, 1907, xci, 212.

[18] Wiens, *Deutsch. Arch. f. klin. Med.*, 1907, xci, 456.

[19] Jochmann and Kantorowicz, *Ztschr. f. klin. Med.*, 1908, lxvi, 153.

The existence of proteolytic enzyme in normal blood serum has long been known. Delezenne and Pozerski[20] have found that blood serum mixed with chloroform acquires the power to digest gelatin and casein. Hedin has shown that the globulin fraction of the blood serum of the ox, obtained by half saturation with ammonium sulphate, contains a weak proteolytic enzyme. The albumin fraction of the serum obtained by complete saturation after removal of globulin contains anti-enzyme, which in normal serum restrains the activity of the enzyme just mentioned. One of us[21] has shown that the anti-enzymotic activity of the blood serum is lost in the presence of 0.2 per cent. acetic acid; in this medium the blood serum exhibits well marked proteolytic activity, but in neutral or alkaline media anti-body balances enzyme, and neither autolysis nor other proteolytic activity is demonstrable.

In order to determine what factors cause changes in the enzymotic and anti-enzymotic activity of the blood serum, a condition which can be produced experimentally offers obvious advantages, for the phenomena which occur can be repeatedly traced from the beginning through various stages of the process. Chloroform poisoning has proved especially favorable for this study because it reproduces the increase of anti-enzyme heretofore frequently noted with the cachexia of carcinoma, with various infections, and with other diseases.

The changes in the liver which accompany prolonged administration of chloroform by inhalation, are clearly defined by the recent studies of Howland and Richards[22] which have directed our attention to this subject. There is necrosis implicating the central part of each hepatic lobule. Disintegration of protein within the body is shown by increased elimination of nitrogen and sulphur by the urine. When intoxication is not severe, there is, instead of necrosis, fatty degeneration of the liver, kidneys, heart muscle, etc. Doyon and Billet[23] have found that the blood may become incoagulable and that incoagulability is associated with diminution of the fibrinogen content of the serum.

[20] Delezenne and Pozerski, *Compt. rend. Soc. de biol.*, 1903, lv, 327, 690, 693.
[21] Opie, *Jour. Exper. Med.*, 1905, vii, 316.
[22] Howland and Richards, *Jour. Exper. Med.*, 1909, xi, 344.
[23] Doyon and Billet, *Compt. rend. Soc. de biol.*, 1905, lviii, 852.

We have followed the coagulability of the blood during the course of chloroform poisoning produced by the daily administration by stomach of a given quantity of chloroform (one or two cubic centimeters per kilogram of body weight), and have found that the coagulation time of the blood increases during the first three days; in some instances the blood becomes wholly incoagulable. Hemorrhage into the gastro-intestinal tract or into the peritoneal cavity is not infrequent, and death often occurs at the end of three or four days. When poisoning is not acutely fatal, a reaction occurs; the coagulability of the blood rapidly regains its former activity, and even though the same daily dose of chloroform is administered, coagulation at the end of about a week after the beginning of the experiment may have become somewhat more rapid than normal. An animal which has survived the critical period of three or four days may live ten or twelve days. The animal has established some form of resistance to the poison. In such instances there is widespread fatty degeneration of the liver and other organs and comparatively little necrosis.

The phenomena which have been described have a close analogy in phosphorus poisoning. There is, it is well known, intense fatty degeneration of the liver with some necrosis, and, in some instances, as Jacoby[24] has shown, diminution of the coagulability of the blood is associated with disappearance of fibrinogen. Furthermore, Jacoby has found that the liver with phosphorus poisoning undergoes more rapid autolysis than normal liver. He suggests that enzyme set free by the liver dissolves the fibrinogen of the blood. Our observations, mentioned above, suggest that changes which occur in the blood as the effect of chloroform or phosphorus may be subject to considerable variation, dependent upon the severity of intoxication and upon the length of the period of administration.

Gradual increase of anti-enzyme for a proteolytic enzyme, namely, leucoprotease, during the progress of intoxication with chloroform and phosphorus, noted at the beginning of our experiments, has suggested the possibility that disintegrating liver tissue may set free those proteolytic enzymes which are demonstrable in normal liver. The attempt has been made to determine if at any stage of intoxi-

[24] Jacoby, *Ztschr. f. physiol. Chem.*, 1900, xxx, 174.

cation the serum exhibits increased proteolytic activity. The enzymotic content of the serum has been tested by incubating a fixed quantity of blood serum during four or five days at 37° C. and subsequently determining the degree of autolysis, or by incubating a fixed quantity of serum with a suitable protein substrate, such as heated blood serum. That hepatic enzyme, which is most readily demonstrated, digests in the presence of acid. To determine if the similar enzyme which exists in the blood undergoes any change during the progress of chloroform intoxication, a series of experiments has been performed on dogs.

A fixed quantity of blood has been subjected to autolysis in the presence of 0.2 per cent. acetic acid. Blood has been drawn at intervals of three or four days by means of a needle inserted through the skin into the jugular vein. A measured amount of serum obtained by centrifugalization of the whipped blood has been diluted with salt solution (0.85 per cent.) and acetic acid in such proportion that the final volume of twenty-five cubic centimeters contains 0.2 per cent. acetic acid. In some instances blood serum denaturalized by heat has been added to the mixture as substrate for the proteolytic enzyme of the serum. All mixtures have been incubated during four or five days at 37° C. A mixture containing the same ingredients has served as control and has been immediately heated to boiling. Since it is undesirable to withdraw repeatedly considerable quantities of blood, the quantity of serum which has been subjected to autolysis has been small and occasionally, for lack of material (*e. g.*, Experiment 9), it has been necessary to omit this control. After coagulation by heat, the coagulum has been removed by filtration, and nitrogen in the filtrate has been determined by the Kjeldahl method; the amount of nitrogen liberated by digestion of coagulable protein will be expressed in cubic centimeters of one-tenth normal sulphuric acid.

In some experiments autolytic activity of serum from an animal receiving daily doses of chloroform has been tested on different days; the first test, made before administration of chloroform, serves as a normal standard for comparison with subsequent changes. In other instances the serum of animals which have received chloroform has been compared with normal serum from another animal.

Repeated tests, some of which will be recorded, have demonstrated that autolysis in neutral or alkaline media under conditions otherwise similar to those just described fails to occur or is much less marked than autolysis in the presence of acid.

EXPERIMENT 1.—A dog (weight 6,950 grm.) received on two successive days 10 c.c. of chloroform. Coagulation time of the blood before administration of chloroform was four minutes and five seconds. On the third day of the experiment the animal was sick and the blood failed to coagulate within one hour. The following figures represent autolysis in the presence of acid before and after administration of chloroform. Furthermore, serum obtained on the third day has been compared with the serum of a normal dog.

	Autolysis of 5 c.c. of serum during 4 days at 37°.			
	Control.	In neutral medium.	0.2 per cent. o-dium carbonate.	0.2 per cent. acetic acid.
Serum of chloroform dog before administration of chloroform. (First day; normal serum.).....	1.35			3.0
Serum of chloroform dog on third day.........................	1.5	1.7	2.5	6.45
Serum of normal dog............	1.75	1.75	2.05	3.6

The accuracy of the figures obtained with serum of the dog which had received chloroform (3d day) and normal serum, autolyzed in the presence of acid, is confirmed by an additional test made with 3 c.c. of each serum tested in the same medium: serum of chloroform dog (3d day), 4.3; normal serum, 2.8.

The administration of chloroform has caused well marked increase in the autolytic activity of blood serum tested in acid, and little change when tested in alkaline (perhaps trivial increase) or in neutral medium.

Since the quantities subjected to autolysis are small, experiments have been multiplied with the purpose of establishing the accuracy of the result. In Experiment 2, in addition to the tests previously employed, serum and protein substrate (denaturalized serum) have been incubated in the presence of acetic acid.

EXPERIMENT 2.—A dog (weight 6,750 grm.) received on four successive days 10 c.c. of chloroform. Blood was drawn on the first day (coagulation time three minutes, forty seconds), on the fourth day (five minutes, twenty-one seconds), and on the fifth day (four minutes, twenty-three seconds).

| | Autolysis of 3 c.c. of serum during 4 days at 37°. | | | 1 c.c. serum, substrate, and 0.2 per cent. acetic acid. |
	Control.	In neutral medium.	0.2 per cent. acetic acid.	
1st day (normal)............	1.0	1.05	3.7	2.85
4th day....................	1.1	1.05	5.05	3.45
5th day....................	1.8	1.75	4.95	4.15

EXPERIMENT 3.—A dog received chloroform by mouth on fourteen successive days. The serum was tested as follows:

| | Autolysis of 3 c.c. of serum during 5 days at 37°. | | |
	Control.	In neutral medium.	0.2 per cent. acetic acid.
1st day (normal)	1.2	— ・	2.4
14th day	—*	1.9	5.65

* Control on fourteenth day doubtless approximates closely digestion in neutral medium (1.9 c.c.) and certainly does not exceed this figure.

EXPERIMENT 4.—A dog (weight 7,000 grm.) received on four successive days 10 c.c. of chloroform. Blood was drawn on the first day (coagulation time, four minutes), on the fourth day (nine minutes, twenty seconds), and on the fifth day (six minutes, twenty-one seconds). The animal was moribund on the fifth day.

| | Autolysis of 3 c.c. serum during 5 days at 37°. | | | 1 c.c. serum, substrate, and 0.2 per cent. acetic acid. |
	Control.	In neutral medium.	0.2 per cent. acetic acid.	
1st day (normal)..............	[1.05]*	1.05	3.2	2.65
4th day	2.1	1.9	4.6	4.3
5th day	5.85	5.9	9.2	8.15

* This control was not determined, as the available serum was insufficient; other experiments show that normal serum incubated in neutral medium gives the same figure as serum coagulated immediately. It has been assumed that this control is identical with the figure obtained after incubation with reaction unchanged.

In Experiment 4, increase of the control is noteworthy; the serum contains, in greatly increased quantity, nitrogenous substances incoagulable by heat. Nevertheless, when these controls are subtracted from the figures obtained after incubation, evidence of increased proteolytic activity is obtained.

In the following experiments, which confirm those recorded

above, there is moderate increase of proteolytic activity, which reaches a maximum on the fourth day of chloroform intoxication.

EXPERIMENT 5.—A dog (weight 5,000 grm.) received on six successive days 10 c.c. of chloroform. Blood was drawn on the first day (coagulation time, one minute, twenty-seven seconds), on the fourth day (coagulation time, eight minutes, fifteen seconds), and on the seventh day (coagulation time, six minutes, six seconds).

| | Autolysis of 3 c.c. serum during 4 days at 37°. | | | 3 c.c. serum, substrate, and 0.2 per cent. acetic acid. |
	Control.	In neutral medium.	0.2 per cent. acetic acid.	
1st day (normal)	0.9	1.05	2.8	2.55
4th day	0.9	1.0	3.7	2.7
7th day	1.0	1.05	3.7	2.85

EXPERIMENT 6.—A dog (weight 4,750 grm.) received on ten out of eleven days (sixth day excepted) 7 c.c. of chloroform. Coagulation time: first day, five minutes, eighteen seconds; fourth day, four minutes, thirty-four seconds; seventh day, two minutes, seven seconds; tenth day, three minutes, fifteen seconds.

| | Autolysis of 4 c.c. serum during 4 days at 37°. | |
	Control.	0.2 per cent. acetic acid.
1st day (normal)	1.2	3.15
4th day	1.2	3.95
7th day	1.5	3.75
10th day	1.5	4.5

EXPERIMENT 7.—A dog (weight 5,600 grm.) received on six out of seven days (none on sixth day) 7 c.c. of chloroform. Coagulation time: first day, five minutes, thirty-one seconds; fourth day, seven minutes, seventeen seconds; seventh day, seven minutes, fifteen seconds.

| | Autolysis of 4 c.c. serum during 5 days at 37°. | |
	Control.	0.2 per cent. acetic acid.
1st day (normal)	1.2	3.45
4th day	1.45	4.85
7th day	1.55	4.35

The experiments demonstrate that the well marked increase of proteolytic activity exhibited by animals which have received chloroform, attains its maximum on the third or fourth day of intoxication, and tends to be greatest when intoxication is most severe and when delay of coagulation time is considerable. Increased activity of autolysis may be absent when the critical period of intoxication

has passed or when the injurious action of chloroform has been slight. In the following experiment there has been no evidence of increased enzymotic activity.

EXPERIMENT 8.—A dog received 10 c.c. of chloroform on six successive days. Coagulation time: first day, three minutes, forty-five seconds; fourth day, ten minutes, sixteen seconds; seventh day, two minutes, twelve seconds.

| | Autolysis of 3 c.c. serum during 4 days at 37°. | | | 3 c.c. serum, substrate, and o.2 per cent. acetic acid. |
	Control.	In neutral medium.	o.2 per cent. acetic acid.	
1st day (normal)................	1.0	1.1	4.0	2.85
4th day	1.15	1.5	4.0	3.0
7th day	2.5	2.4	3.9	3.8

The very active anti-enzymotic activity of this serum will be mentioned later.

In the following experiment there is a close relationship between proteolytic activity and severity of intoxication, well indicated by delay of coagulation time.

EXPERIMENT 9.—A small quantity of serum (1 c.c.) has been allowed to act upon coagulated protein (5 c.c. of heated blood serum) during five days at 37° C. The sera of four dogs which have received 7 c.c. of chloroform on two successive days have been drawn on the third day, and the proteolytic activity in neutral and acid media compared with that of the sera from two normal dogs.

| | Coagulation time. | | Serum + heated serum after 5 days at 37°. | Serum + heated serum + o.2 per cent. acetic acid after 5 days at 37°. |
	Minutes.	Seconds.		
Normal Dog A...........	1	15	1.7	1.65
Normal Dog B...........	2	30	1.75	1.75
Chloroform Dog A........	7	30	1.85	2.1
Chloroform Dog B........	9	30	1.65	2.15
Chloroform Dog C........	16	30	2.35	2.25
Chloroform Dog D	30	0	2.65	3.55

On the fourth day Chloroform Dog C was much less sick than on the previous day, whereas Chloroform Dog D was moribund. The following data were obtained:

| | Coagulation time. | | Serum + heated serum after 5 days at 37°. | Serum + heated serum + o.2 per cent. acetic acid after 5 days at 37°. |
	Minutes.	Seconds.		
Chloroform Dog C........	12	30	1.6	2.3
Chloroform Dog D	21	0	2.8	4.45

The figures obtained are small, but the changes which occur are the same as those previously described. It is noteworthy that the coagulation time of the blood is a fair index of the intoxication caused by chloroform. There has been a close relationship between coagulation time and autolytic activity, both in an acid and in an approximately neutral medium. The close relation between the toxic effect of chloroform and autolytic activity of the blood serum is well illustrated by the tests made on the fourth day. Dog C has in part recovered from the depression produced by the first two doses, and its serum exhibits autolytic activity not far removed from normal, whereas the serum of Dog D, which has been profoundly poisoned, exhibits increased autolytic activity.

Having in view the possibility that degenerative changes in the liver are associated with increased activity of proteolytic enzymes, we have undertaken a series of experiments to determine if the blood serum of animals receiving chloroform or phosphorus exhibits any alteration of the normal power of blood serum to restrain the autolysis of liver.

EXPERIMENT 10.—A dog has been given daily doses of chloroform (2 grm. per kilo of body weight) during ten days. An emulsion has been prepared by mixing finely ground liver with four times its volume of salt solution; 20 c.c. of this mixture have been allowed to undergo autolysis during five days at 37° C. both alone and in the presence of blood serum from the same animal and from a normal animal. The volume of the mixture has been increased in every instance to 25 c.c. Autolysis of liver alone is represented by 25.7 c.c. N/10 sulphuric acid.

	Normal serum.	Chloroform serum.
Liver + 1 c.c. serum	22.25	20.2
Liver + 2.5 c.c. serum	18.4	18.65
Liver + 5 c.c. serum	15.5	17.05

Blood serum has inhibited autolysis in slight degree, but no noteworthy change of inhibition has been caused by administration of chloroform. When a small quantity of serum has been employed, inhibition has been slightly greater with chloroform than with normal serum. It is probable that the slightly higher figure obtained with five cubic centimeters of serum of the animal which received chloroform, is referable to its greater content of nitrogenous substances incoagulable by heat.

In other experiments, fresh liver of the rabbit has been subjected to autolysis in the presence of serum of a normal rabbit and of serum of a rabbit poisoned with phosphorus.

EXPERIMENT 11.—A suspension of normal rabbit's liver (10 c.c.) has undergone autolysis represented by 5.95 c.c. N/10 sulphuric acid (control, 2.05 c.c.). The following figures represent the effect of normal serum and of serum of a rabbit receiving phosphorus upon the same quantity of this suspension:

	Normal serum.	Chloroform serum.
Liver + 0.25 c.c.	6.1	6.0
Liver + 0.5 c.c.	5.45	6.05
Liver + 1 c.c.	5.65	5.1

There has been no constant or noteworthy difference in the effect of normal serum and of serum after administration of phosphorus upon autolysis of normal liver.

In a subsequent experiment the liver of an animal which has received phosphorus has been allowed to undergo autolysis. The attempt has been made to determine if serum of an animal similarly treated with phosphorus differs from normal serum in its effect upon the autolysis of phosphorus liver.

EXPERIMENT 12.—One gram of liver exhibiting fatty degeneration as the result of phosphorus has been suspended in 25 c.c. of salt solution and allowed to autolyze alone and in the presence of serum.

	Control.	After incubation during 5 days at 37°.	Digestion.
Liver........................	2.6	6.05	3.45
Liver +2 c.c normal serum.......	3.1	5.05	1.95
Liver +2 c.c. phosphorus serum...	3.7	6.45	2.75

Both normal and phosphorus serum have inhibited autolysis of the liver of an animal which has received phosphorus. Inhibition has been less with the serum obtained after administration of phosphorus than with that of the normal animal, but the foregoing experiments offer little evidence that the anti-enzymotic activity of the phosphorus animals undergoes any significant change.

Far more constant results have been obtained when the serum of animals which have received chloroform has been allowed to act upon an enzyme which, unlike the autolytic enzyme of the liver,

acts with greatest activity in an alkaline medium. Various quantities of serum from animals which have received chloroform and of serum from normal animals have been allowed to act upon a fixed quantity of enzyme of polynuclear leucocytes (leucoprotease).

Leucoprotease (twenty milligrams) has been allowed to digest coagulated blood serum both alone and in the presence of normal and of chloroform serum.

EXPERIMENT 13.—The serum of a dog which has received 160 c.c. of chloroform during fourteen days has been employed.

	Control.	After incubation during 5 days at 37°.
20 mgr. leucoprotease + coagulated serum	1.55	19.4

	Normal serum.	Chloroform serum
Above mixture + 0.25 c.c. serum	19.25	12.1
Above mixture + 0.5 c.c. serum	15.55	4.35
Above mixture + 1.0 c.c. serum	5.45	3.3

Inhibition has been uniformly greater with the serum of the animal which has received chloroform than with the serum of a normal animal.

The experiment has been repeated with serum of a dog which has received repeated doses of chloroform administered by mouth.

EXPERIMENT 14.—

	Control.	After incubation during 5 days at 37°.
20 mgr. leucoprotease + coagulated serum	1.9	20.35

	Normal serum.	Chloroform serum.
Above mixture + 0.25 c.c. serum	20.3	15.4
Above mixture + 0.5 c.c. serum	19.1	4.0
Above mixture + 1.0 c.c. serum	8.8	3.2

Small quantities (0.2 and 0.5 cubic centimeter) of normal serum produce almost no effect upon the enzyme, yet the same quantities of chloroform serum greatly diminish its activity.

Continued increase of anti-enzymotic activity of the blood serum is well seen in the following experiment in which tests repeated at intervals of three days have been made with the serum of an animal to which chloroform has been administered daily.

EXPERIMENT 15.—A dog weighing 4,750 grm. has received daily during eleven days (the sixth day excepted) 12 c.c. of chloroform. From 25 to 35 c.c. of

blood have been drawn at intervals of three days. Coagulation time: first day, five minutes, eighteen seconds; fourth day, four minutes, forty-three seconds; seventh day, two minutes, seven seconds; tenth day, three minutes, fifteen seconds. On the fifth day there has been slight jaundice which has subsequently increased in intensity. The inhibition of 20 mgr. leucoprotease caused by 0.5 c.c. of serum is shown by the following figures:

	After incubation during 5 days at 37°.
20 mgr. leucoprotease + coagulated blood serum	17.25
Same mixture + 0.5 c.c. serum on 1st day (normal)	13.75
Same mixture + 0.5 c.c. serum on 4th day	12.45
Same mixture + 0.5 c.c. serum on 7th day	5.25
Same mixture + 0.5 c.c. serum on 10th day	2.9

The following test shows that administration of phosphorus has the same effect as administration of chloroform upon the anti-enzymotic activity of the blood serum.

EXPERIMENT 16.—A dog weighing 7,700 grm. has received 1/50 grain of phosphorus every second day during fourteen days. Its blood serum (coagulation time, four minutes, nine seconds) has been compared with two normal sera.

	After incubation during 5 days at 37°.
20 mgr. leucoprotease + coagulated serum	17.25
Same mixture and 0.5 c.c. normal serum A	13.75
Same mixture and 0.5 c.c. normal serum B.	15.15
Same mixture and 0.5 c.c. phosphorus serum	7.3

One-half cubic centimeter of normal serum exhibits slight anti-enzymotic activity, whereas the same amount of serum from an animal which has received phosphorus causes strong inhibition of an equal amount of enzyme.

Increased inhibition of leucoprotease, demonstrable in the serum of dogs which have received chloroform or phosphorus, has been found in rabbits as well.

EXPERIMENT 17.—In the following series of tests, sera from a rabbit which has received phosphorus has been obtained on different days and has been compared with normal sera.

20 mgr. leucoprotease + coagulated blood serum: control, 2.8; after incubation during 5 days at 37° C., 19.45.

With serum of the animal which has received small doses of phosphorus, digestion caused by leucoprotease is constantly less than with normal sera.

		Normal serum.	Phosphorus serum.
A	Same mixture + 0.25 c.c. serum	15.2	10.75
	Same mixture + 0.5 c.c. serum	7.05	5.2

The following test has been made 4 days later:

| B | Same mixture + 0.25 c.c. serum .... | 16.95 | 10.9 |

The following test has been made one day after last:

| C | Same mixture + 0.25 c.c. serum .... | 16.55 | 11.95 |
| | Same mixture + 0.5 c.c. serum .... | 7.25 | 4.6 |

The foregoing experiments show that the blood serum under the influence of a poison such as chloroform, acting with intensity sufficient to cause necrosis of the liver and diminish the coagulability of the blood, acquires increased ability to cause digestion of protein. This increased proteolysis is, in part at least, referable to an enzyme which, like the autolytic enzyme of the liver and almost all other organs, digests with maximum activity in a weakly acid medium. It is not improbable that proteolytic enzyme is liberated by disintegration of parenchymatous cells and carried away by the blood.

In animals which survive the severe intoxication produced by large doses of chloroform, or in those which have repeatedly received smaller doses, the blood serum acquires increased ability to restrain the action of a proteolytic enzyme; but this anti-enzymotic action is exerted, not upon the enzyme of the liver, which acts in acid, but upon an enzyme which acts in the presence of alkali, namely, leucoprotease. There is little reason to doubt that the same serum will restrain the action of a second enzyme which acts in alkali, namely, trypsin, for, on the one hand, Jochmann and Kantorowicz have found that anti-enzyme for enzyme of leucocytes and for trypsin increases simultaneously, and, on the other hand, K. Meyer has found the anti-tryptic activity of the serum increased in animals which have received phosphorus. Anti-enzyme for one type of enzyme is increased; whereas evidence heretofore available indicates that a second type of enzyme is predominant in those tissues which are subject to the destructive action of the poison. Furthermore, our experiments have shown that an enzyme similar to that of the injured organ accumulates in the blood serum. It is difficult to correlate these observations.

Nevertheless, the experiments of Hedin and of Dochez show that

the liver contains a second enzyme which, unlike that directly obtainable from fresh liver, digests protein in the presence of an alkaline medium. These observations suggest the possibility that this enzyme may have a part in changes which increase the antienzymotic activity of the blood serum after administration of substances which injure the liver. Treatment of fresh liver with acid discloses an enzyme which digests in the presence of acid. Does the serum of an animal which has received repeated doses of chloroform and exhibits increased power to inhibit leucoprotease and, doubtless, trypsin, exhibit as well increased power to inhibit that enzyme of the liver which is similar to these two enzymes? The experiments which follow have been undertaken with the purpose of answering this question.

Using the method described by one of us (Dochez), we obtained that enzyme which digests protein in the presence of alkali, by subjecting fresh liver to the action of weak acetic acid during a period of twenty-four hours. Liver just after removal with aseptic precautions from an etherized animal has been finely ground by means of a hashing machine; ground liver has been mixed with twice its volume of salt solution to which acetic acid has been added in such amount that the concentration of the mixture is 0.2 per cent. After this mixture has stood at a temperature slightly above freezing during twenty-four hours, the acid has been neutralized by an equivalent quantity of one-tenth normal sodium hydroxide. Ten cubic centimeters of this neutralized mixture has been allowed to undergo autolysis and the inhibition caused by various quantities of serum has been tested. In order to determine if the inhibitory activity of serum is increased during the progress of chloroform poisoning, normal serum has been compared with serum obtained after repeated administration of chloroform. For the purpose of the present investigation, a comparative study of anti-enzymotic activity for leucoprotease and for enzyme of liver has been essential, and parallel tests of the action of each serum upon the two enzymes have been made.

The change which occurs rapidly when liver is treated with acid occurs slowly when liver in approximately neutral suspension is preserved under conditions which prevent bacterial growth. In the

following experiment a suspension allowed to stand under toluol during two months has been used.

EXPERIMENT 18.—Two dogs, Dogs A and B, have received daily 10 c.c. of chloroform by stomach. Blood has been drawn on the first and on the fourth day of the experiment. Anti-enzymotic activity of this serum has been tested by allowing a given quantity of leucoprotease (20 mgr.) to act upon substrate (5 c.c. heated blood serum) in the presence of 0.5 c.c. of blood serum obtained before administration of chloroform and on the fourth day of administration. A parallel test has been made with liver which has been allowed to stand on ice over toluol during two months in order to liberate that enzyme which, like leuco-protease, digests in alkali. This suspension of liver has been made by mixing equal volumes of liver, ground through a hashing machine, and of salt solution with sodium carbonate in the proportion of 0.1 per cent. Ten cubic centi-meters of this suspension diluted with salt solution so that the total volume is 25 c.c. has been allowed to undergo autolysis; the same quantity has been autolyzed in the presence of blood serum.

Leucoprotease (20 mgr.) acting upon the substrate is represented by 16.2 c.c. N/10 H_2SO_4 (control, 1.8 c.c.). Autolysis of liver (10 c.c.) alone is represented by 7.5 c.c. N/10 H_2SO_4 (control 3.3 c.c.). The following table represents a com-parison of digestion by leucoprotease and autolysis of liver in the presence of the same serum.

	Digestion with leucoprotease + 0.5 c.c. serum.	Autolysis of active liver.	
		+ 0.5 c.c. serum.	+ 1 c.c. serum.
CHLOROFORM DOG A.			
With 0.5 c.c. serum obtained before administration of chloroform ...	13.6	6.7*	8.15
With 0.5 c.c. serum obtained on 4th day of chloroform administration	3.35	5.2	—
CHLOROFORM DOG B.			
With 0.5 c.c. serum obtained before administration of chloroform ...	11.4	8.35	7.55
With 0.5 c.c. serum obtained on 4th day of chloroform administration	3.15	5.3	5.45

* This figure should be somewhat higher, since loss occurred as result of accident during Kjeldahl determination.

On the fifth day of the experiment the serum of each dog has been again tested, but as it has been necessary to use for autolysis a different suspension of liver, no comparison with the foregoing test is possible and consequently the two sera have been compared with sera from two normal dogs. Liver suspended in an equal volume of salt solution has been allowed to stand in the ice-chest under toluol during approximately two months; slight acidity of the suspension has been neutralized by addition of N/10 NaOH (4.8 c.c. to 170 c.c. of suspension). Ten cubic centimeters of the suspension have been used for each test; 5 c.c. of blood serum have been added with the purpose of supplying additional substrate for the proteolytic enzyme derived from the liver.

Digestion after incubation during five days at 37° C. is represented by 12.95 c.c. N/10 H$_2$SO$_4$ (control 4.8 c.c.).

	With 0.5 c.c. serum.	With 1 c.c. serum.
Serum of Chloroform Dog A	7.65	7.1
Serum of Chloroform Dog B	7.6	5.65
Serum of Normal Dog A	9.65	6.95
Serum of Normal Dog B	8.2	7.3

The degree of inhibition will obviously depend upon the strength of the enzyme and of the anti-enzymotic activity of the serum. In the second series of tests, one cubic centimeter of normal serum has so materially reduced the activity of the enzyme that differences between normal serum and serum after administration of chloroform are in large part obliterated.

In the first series of tests (Experiment 18), the inhibiting action of each serum upon leucoprotease has been compared with its action upon enzyme of liver; with well marked decrease of anti-enzyme for the one, there has been a parallel increase for the other. In the following experiment the serum tested has shown no noteworthy decrease of anti-enzyme for leucoprotease and there has been little, if any, change in its ability to restrain the similar enzyme of liver.

EXPERIMENT 19.—Two animals received on four successive days 10 c.c. of chloroform. Blood was drawn before administration and on the fourth day of the experiment. The effect of these sera upon leucoprotease and upon enzyme of liver is shown by the following figures:

Leucoprotease (20 mgr.) acting upon substrate is represented by 16.2 c.c. N/10 H$_2$SO$_4$ (control, 1.8 c.c.). Autolysis of liver alone is represented by 7.5 c.c. N/10 H$_2$SO$_4$ (control, 3.3 c.c.).

Chloroform Dog C.

	Digestion with leuco-protease + 0.5 c.c. serum.	Autolysis of active liver + 0.5 c.c. serum.
With serum obtained before administration of chloroform	9.5	7.9
With serum obtained on 4th day after chloroform administration	8.5	7.55

Chloroform Dog D.

With serum obtained before administration of chloroform	14.2	7.75
With serum obtained on 4th day of chloroform administration	13.7	7.85

In the following experiment serum of an animal which has received chloroform is compared with that of a normal dog.

EXPERIMENT 20.—A dog weighing 21 kilograms has received daily doses of chloroform increasing from 0.5 to 7 c.c. Blood has been drawn on the fourteenth day of the experiment. Inhibition of leucoprotease and of enzyme of liver are compared.

Leucoprotease (20 mgr.) acting on substrate causes proteolysis represented by 16.6 c.c. N/10 H_2SO_4, control being 1.5. Autolysis of the liver employed is represented by 27.2 c.c. N/10 H_2SO_4, control being 16.05 c.c. In order to make the degree of inhibition more conspicuous, the control has been subtracted from the figure obtained after digestion during four days at 37° C.

	Total digestion with leucoprotease + 0.5 c.c. serum.	Total autolysis of liver.	
		+ 0.5 c.c. serum.	+ 1 c.c. serum.
With serum of normal dog	14.9	12.7	12.15
With serum of chloroform dog	2.8	8.45	9.75

Well marked increase of anti-enzyme for liver pretreated with acid corresponds with increased anti-enzyme for leucoprotease. In the following experiment, identical in other respects with that just described, the effect upon autolysis of pretreated liver, both of normal serum and of serum from a dog which has received chloroform, has been tested in the presence of alkali.

EXPERIMENT 21.—The foregoing experiment has been repeated by comparing serum of a normal dog with serum of a dog (weighing 21 kilograms) which has received 21 c.c. of chloroform daily (the seventh day excepted) during ten days. The leucoprotease and suspension of liver used in Experiment 20 have been employed and the control has been subtracted from the figure obtained after digestion during four days at 37° C.

	Total digestion with leucoprotease + 0.5 c c. serum.	Total autolysis of liver.	
		+ 0.5 c.c. serum.	+ 1 c.c. serum.
With serum of normal dog	13.2	15.65	13.8
With serum of chloroform dog	4.67	12.9	12.85

In order to obtain further information concerning the progress of the changes which effect the anti-enzymotic activity of the blood serum, comparative tests have been made with leucoprotease and with pretreated liver during the course of prolonged administration of chloroform. The method of testing inhibition is identical with

that previously employed; no alkali has been added to the mixtures prepared for proteolysis.

EXPERIMENT 22.—Serum has been obtained from a dog weighing 9 kilograms, which during nineteen days has received 9 c.c. of chloroform daily. Coagulation time, which has been two minutes and ten seconds before administration of chloroform, has been tested whenever blood has been drawn and has never risen above three minutes and thirty-five seconds. The animal has been jaundiced on the eighteenth day of the experiment.

The following table gives the results of a large series of tests made on different days with various quantities of serum added to mixtures containing either leucoprotease or liver rendered active by pretreatment with acid.

	Proteolysis with leucoprotease.			Autolysis of pretreated liver.				
	With no serum.	0.25 c.c. serum.	0.5 c.c. serum.	With no serum.	0.25 c.c. serum.	0.5 c.c. serum.	1 c.c. serum.	2 c.c. serum.
1st day (normal) .	12.9	11.65	9.5	16.2	12.5	11.2	10.5	8.9
4th day......		7.1	4.45	15.0	9.8	8.45	7.55	.6.9
7th day......		6.5	4.4	18.2	10.1	9.0	8.05	8.0
10th day.....		6.7	5.4	16.6	9.9	9.5	7.6	7.1
14th day.....	15.7	6.2	4.6	16.3	10.5	10.0	8.8	8.1
18th day.....	14.8	4.4	3.4	15.9	9.3	8.5	7.1	7.0

Although there are some irregularities in the progress of the changes represented by the foregoing figures, it is obvious that the power of the serum to inhibit the active enzyme of acid-treated liver exhibits an increase parallel with the increased power to inhibit leucoprotease. Here, as in previous experiments, larger quantities even of normal serum (one or two cubic centimeters) cause such well marked inhibition of the hepatic enzyme that differences are less conspicuous than when small quantities (0.25 or 0.5 cubic centimeter) are employed. To demonstrate more clearly the parallel increase of anti-enzyme for the two enzymes which have been compared, curves (Fig. 1.) have been plotted to represent digestion caused by hepatic enzyme (unbroken line) and leucoprotease (broken line) in the presence of 0.25 and of 0.5 cubic centimeter of blood serum.

The experiments which have been described show that chloroform given in quantity sufficient to produce profound intoxication, indicated by necrosis of the liver and loss of coagulability of the blood, causes an increase of the proteolytic enzyme normally present in the blood serum. Increase of proteolytic activity is exhibited

in the presence of weak acid, but is much less evident in an alkaline or neutral medium. This observation suggests the possibility that the proteolytic enzyme of liver tissue, which causes autolysis and has maximum activity in the presence of acid, is freed by disintegration of hepatic cells and accumulates in the blood serum.
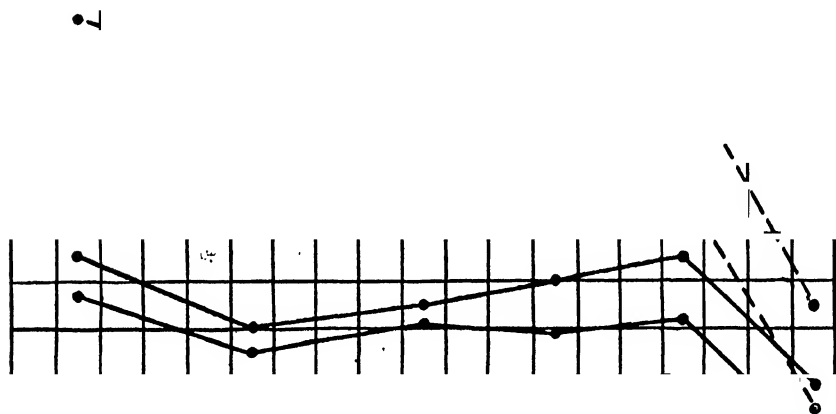

CHART I.—Diagram showing increase of anti-enzymes in the blood serum of an animal (see Experiment 22) receiving chloroform daily. The solid lines represent digestion by enzyme of acid-treated liver in the presence of 0.25 c.c. (upper solid line) and of 0.5 c.c. (lower solid line) of blood serum. The broken lines represent digestion by leucoprotease in the presence of 0.25 c.c. (upper broken line) and of 0.5 c.c. (lower broken line) of blood serum. Changes in the two pairs of lines are an index of the activity of anti-enzyme for the two enzymes, and are, in general, parallel.

The anti-enzymotic action of the blood serum has been tested in animals which have repeatedly received chloroform in quantity insufficient to produce fatal intoxication. There is no increase of the normal power of the serum to restrain autolysis of liver. Nevertheless, when anti-enzymotic action of blood serum is tested

with leucoprotease, gradually increasing activity constantly accompanies continued administration not only of chloroform but of phosphorus as well. On the one hand, it is especially noteworthy that the enzyme of leucocytes digests protein in an alkaline or neutral medium, whereas the proteolytic enzyme of liver exhibits maximum activity in acid. On the other hand, it is now known that treatment of liver with weak acid renders active an enzyme which digests with energy in alkali in much the same way that treatment of fresh extract of pancreas with acid transforms pancreatic zymogen into trypsin. Is increase of anti-enzyme for leucoprotease an index of increase of anti-enzyme for this second enzyme of liver, which, like leucoprotease, digests protein with maximum activity in alkali, and is, perhaps, freed by disintegration of liver cells? Tests with acid-treated liver show that anti-enzyme for the hepatic enzyme which digests in alkali is increased during the progress of chloroform intoxication, and parallel tests with leucoprotease and with this hepatic enzyme have shown that serum which exhibits increased inhibition of one exhibits increased power to restrain the other.

The following scheme represents the relationship of hepatic enzymes to enzymes and anti-enzymes of the serum which is suggested by the foregoing experiments: ·

Proteolytic enzymes of liver.	Proteolytic enzymes of blood serum.	Anti-enzymotic action of blood serum.
(A) Enzyme digesting in acid.	(A) Enzyme digesting in acid. *Increased by chloroform.*	(A) Alkalinity of serum.
(B) Enzyme digesting in alkali. (Made active by acid-treatment of fresh liver.)	(B) Enzyme digesting in alkali. (Present in globulin fraction of serum and inhibited by anti-enzyme of albumin fraction.)	(B) Anti-enzyme for trypsin, leucoprotease, and similar enzyme of liver. *Increased by chloroform.*

Increase of anti-enzyme occurring in association with intoxication by chloroform or by phosphorus is, doubtless, similar to that which has been repeatedly observed with the cachexia of malignant growth

with various infections, and with other conditions. The increased protein disintegration which accompanies such processes is, perhaps, associated with liberation of proteolytic enzymes similar to those concerned in the postmortem autolysis of organs. The experiments which have been described suggest that formation of anti-enzyme is a means by which the body is protected from enzymes liberated by degeneration of cells.

ARTIFICIAL STIMULATION AND INHIBITION OF THE GROWTH OF NORMAL AND SARCOMATOUS TISSUES

A FOURTH ARTICLE ON CULTIVATION OF TISSUE IN VITRO *

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AND

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NEW YORK

We have studied, by the method of cultivation of tissues *in vitro,* the properties acquired by the plasma during the development of an experimental malignant tumor. The experiments were performed on chickens of thoroughbred stock, obtained from the same farm, inoculated with the sarcoma propagated by Dr. Peyton Rous. Fragments of this tumor and of normal tissues of chick embryos and adult individuals were cultivated comparatively in the plasma obtained from normal animals and in the plasma obtained from sarcomatous chickens at different stages of the disease. Sharp differences in the rate and in the dimensions of the growth could easily be observed, even without the use of the microscope.

INFLUENCE OF NORMAL AND SARCOMATOUS PLASMAS ON THE GROWTH OF SARCOMATOUS TISSUES

Sarcoma cultivated in the plasma of the individual bearing the tumor grew very extensively. The area covered by the new cells in twenty-four hours was often fifteen or twenty times larger than the area of the primitive fragment. When the same sarcoma was cultivated in the plasma of a normal animal its growth was active although less extensive than in the plasma of the same individual. The same sarcoma cultivated in the plasma of another sarcomatous animal grew very little or even

did not grow at all. If the plasma was taken from an animal recently inoculated and bearing a small tumor, it inhibited, slightly only, the growth of the tumor, but if it belonged to an animal bearing a very large and actively growing tumor it prevented completely the development of the cultures. Therefore the *plasma of a sarcomatous animal acquires the property of inhibiting the growth of a sarcoma taken from another animal.*

What causes the inhibition? Is it due to substances secreted by the tumor or to substances produced by the organism as a reaction against the tumor? If the inhibition is caused by substances secreted by the tumor and contained in the blood of the sarcomatous animal, it might be possible to give that inhibiting power artificially to normal plasma by adding to it serous extract of sarcoma. Therefore we added a little sarcomatous extract to cultures of sarcoma in normal and in sarcomatous plasmas. The growth of sarcoma in normal plasma with sarcomatous extract was markedly accelerated. It showed, evidently, that the inhibiting power of sarcomatous plasma was not due to substances secreted by the tumor. Then it is, possibly, caused by substances produced by the organism as a reaction against the tumor. It is important to consider that the inhibiting power of the sarcomatous plasma is felt only by a tumor belonging to another animal, that is, by a homogenic tumor. The tumor belonging to the animal from which plasma was taken, that is, the autogenic tumor, is not affected by the inhibiting substances. If a tumor could be sensitized to the action of the inhibiting substances existing in the plasma of the organism on which it grows, its development in the same plasma would probably be prevented.

INFLUENCE OF NORMAL AND SARCOMATOUS PLASMAS ON THE GROWTH OF NORMAL TISSUES OF NORMAL AND SARCOMATOUS ANIMALS

The comparative action of normal and sarcomatous plasmas was studied on normal spleen of sarcomatous animals, on normal spleen of normal adult animals, and on spleen, cornea, cartilage and skin of embryo chicks, eight and fifteen days old.

The plasmatic medium was taken from normal adult animals and from animals bearing small and recent tumors, or old and extensive tumors.

Fragments of spleen extirpated from a normal animal grew sometimes at the same rate in normal and sarcomatous plasmas, but it also happened very often that they grew more quickly and more extensively in sarcomatous plasma.

Fragments of spleen extirpated from a sarcomatous animal grew almost always much more quickly and extensively in sarcomatous plasma than in normal plasma.

It was also observed that spleen taken from chick embryos grew very much better in sarcomatous than in normal plasma. Embryonic connective tissue also showed a larger development in sarcomatous plasma.

The activation of growth produced by the sarcomatous plasma is affected in a large measure by the size and the age of the tumor of the animal which gave the plasma. Plasma from an animal with a large and old tumor is more stimulating for normal tissues than the plasma from an animal with a small and recent tumor.

What causes the stimulating action of sarcomatous plasmas on normal tissues, and especially on spleen?

It may be substances secreted by the tumor and diverted into the blood. In some experiments the growth of normal tissues cultivated together with fragments of tumor in normal plasma was accelerated. The results were not constant but showed that sarcoma may stimulate the growth of a tissue under certain ill-determined conditions. Therefore we attempted to modify the action of normal plasma by adding to it a little serous extract of sarcoma. Several series of experiments of this kind were performed. Normal spleen was cultivated in normal and sarcomatous plasma. In one of the series a little sarcomatous extract was added to normal and sarcomatous plasma. In the series where no extract was added, spleen grew very much better in sarcomatous than in normal plasma. On the contrary, in the series in which extract was added spleen grew more quickly and more extensively in normal plasma. It showed that normal plasma had become stimulating by the mere addition of a little sarcomatous extract. The same experiments were repeated with embryonal spleen. Embryonal spleen was exceedingly sensitive to the action of the extract. We observed a very marked and sometimes an enormous acceleration of the growth. In one case, the area covered in twenty-

seven hours by the new cells was almost forty times
larger than the area of the primitive fragment. In a
few days the primitive fragment resolved itself into
cells and disappeared almost completely. Therefore, the
stimulating influence for normal spleen acquired by the
sarcomatous plasma may be due to a substance produced
by the tumor and analogous possibly to the serous
extract of sarcoma.

Is the stimulating substance specific to sarcoma, or is
it contained in all actively growing tissues of connective
and other types? We had already observed that the
growth of cartilage can be stimulated by a fragment of
quickly growing spleen, and that a new culture of spleen
is activated by the addition of a little piece of spleen in
full growth. Nevertheless, fragments of spleen cul-
tivated in plasma with tumor and embryonal extract
showed that tumor extract has a very much more ener-
getic stimulating power than embryonal tissue extract.

These biologic characteristics of sarcoma have been
studied only on the tumor of Dr. Rous. It will be
important to ascertain whether they apply only to this
sarcoma, or if they express a more general law of devel-
opment of experimental malignant tumors.

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Über das Vorkommen der Altmannschen Granulationen in den weissen Blutzellen.

Von

E. E. Butterfield[1], **Albert Heineke** und **Erich Meyer.**

Hierzu die lithograph. Tafel Nr. VI.

In den letzten Jahren haben sich die morphologischen Untersuchungen auf folgende zwei Fragen konzentriert:

1. Auf die Deutung der myeloiden Umwandlung der hämatopoetischen Organe, und

2. auf die Beziehung der grossen Lymphozyten („Grosslymphozyten" Pappenheim) zu den grossen ungranulierten Vorstufen der Myelozyten [grosse „Lymphoidzellen" (Michaelis und Wolff) „Myeloblasten" Naegeli, Schridde]. Diese beiden Fragen stehen in untrennbarem Zusammenhang miteinander, denn die Auffassung der nichthämoglobinhaltigen ungranulierten grossen Zellen, die im myeloiden Gewebe neben den Myelozyten gefunden werden, wird bestimmt durch die Stellung, die man diesen Zellen im Stammbaum der Knochenmarkszellen oder Lymphozyten zuteilt.

Wir haben in früheren Arbeiten gezeigt — und diese Auffassung ist allgemein angenommen worden — dass die pathologisch anatomischen Veränderungen der beiden Leukämieformen zwar prinzipiell verschiedener Natur sind, haben es aber mit gutem Grund vermieden, uns bindend über die Mutterzellen der beiden Zellsysteme zu äussern; denn die von Naegeli und Schridde[2] angegebenen Argumente zur Unterscheidung der Lymphoblasten und Myeloblasten erschienen uns keineswegs beweisend, wir selbst aber konnten keine neuen Kriterien zur Unterscheidung beibringen. Deshalb schlossen wir uns anfangs in der Nomenklatur dem nichts präjudizierenden Ausdruck „Lymphoidzellen" an.[3] In einer gründ-

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[1] Fellow of the Rockefeller Institute for Medical Research New York.

[2] s. die Literatur bei Naegeli, Blutkrankheiten und Blutdiagnostik 1907, und bei Ehrlich, Lazarus, Die Anämie, 2. Aufl., I. Abteil., I. Teil besorgt von Lazarus und Naegeli 1909.

[3] Erich Meyer und Albert Heineke, Über Blutbildung bei schweren Anämien und Leukämien. Deutsch. Arch. f. klin. Med., Bd. LXXXVIII.

lichen Durcharbeitung unseres gesamten damaligen Materials,[1] das sich auf 18 Fälle von myeloider Leukämie und Anämie mit myeloider Umwandlung bezog, konnte dann der eine Punkt der Naegelischen Myeloblastenlehre widerlegt werden, indem gezeigt wurde, dass die Zahl der Kernkörperchen bei den in Frage kommenden Zellen je nach dem Funktionszustand wechselt, und dass daher nach der Zahl der Kernkörperchen kein prinzipieller Unterschied zwischen Lymphozyten und Myeloblasten erblickt werden darf. Es soll gar nicht in Abrede gestellt werden, dass im allgemeinen die typischen kleinen Lymphozyten ein bis zwei Kernkörperchen besitzen, wir müssen es aber bestreiten, dass es immer Zellen der myeloiden Reihe seien, die mehrere Kernkörperchen aufweisen; denn einerseits haben die meisten ungranulierten Vorstufen der Myelozyten in unseren Fällen auch nur ein bis zwei Kernkörperchen, und andrerseits kommen gerade den Zellen der Keimzentren mehrere Kernkörperchen zu. Es konnte weiter gezeigt werden, dass es Fälle gibt, die nach dem Verhalten des Blutbildes den akuten lymphatischen Leukämien (Grosslymphozyten) entsprechen und bei denen die Organuntersuchung eine Unterscheidung von akuter myeloider Leukämie nicht zulässt. Den Schluss, dass die Vorstufen der Myelozyten (Myeloblasten) identisch seien mit den Vorstufen der kleinen Lymphozyten haben wir absichtlich nicht gezogen, es ging vielmehr aus diesen Untersuchungen nur hervor, dass die sogenannten „Myeloblasten" dieser Fälle mit grösster Wahrscheinlichkeit identisch seien mit den Zellen, die man bisher als „grosse Lymphozyten" (Grosslymphozyten) bezeichnet hat. Der Beweis, dass dies wirklich so ist, wird in der folgenden Arbeit geliefert werden; denn es wird gezeigt, dass auch bezüglich der Altmannschen Granulationen keine Unterscheidung möglich ist.

Wie wir aber bereits zweimal hervorgehoben haben,[2] soll damit gar nichts über die Mutterzellen der kleinen Lymphozyten, als die man ja gewöhnlich die Keimzentrumszellen ansieht, ausgesagt sein. Es sei nochmals darauf hingewiesen, dass ein Beweis für die Identität dieser Zellen mit den grossen Lymphozyten der Hämatologen bisher nicht erbracht worden ist. Das bisherige Endresultat aller unserer Zelluntersuchungen in dieser Richtung ist nur das eine: die ungranulierten grossen Zellen der sogenannten akuten Leukämie, die man als grosse Lymphozyten bezeichnet hat, sind identisch mit den Myeloblasten. Der Ausdruck grosse Lymphozyten, dem eine nicht bewiesene Beziehung zu den echten kleinen Lymphozyten untrennbar anhaftet, sollte unserer Meinung nach für diejenigen Fälle ausgeschaltet werden, in denen nicht wirkliche Übergänge zu kleinen Lymphozyten nachgewiesen werden können. Wenn im Verlaufe chronisch myeloider Leukämien „grosse Lymphozyten" oder „Lymphoidzellen" in vermehrter Menge auftreten, so dürften diese nichts anderes bedeuten, als die fast immer bei diesen Fällen vorhandenen „lym-

[1] Butterfield, Über die ungranulierten Vorstufen der Myelozyten und Bildung in Milz, Leber und Lymphdrüsen, ebenda Bd. XCII.

[2] Vergl. Butterfield l. c. und Erich Meyer: Weitere Untersuchungen über extrauterine Blutbildung. Münch. med. Wochenschrift 1908, Nr. 22.

phoiden Markzellen" (Türk), da diese Zellen alle entweder normale oder schlecht differenzierte Vorstufen der Myelozyten sind und somit zur myeloiden Zellreihe gehören. Die in einigen von Türk beschriebenen Fällen sub finem morbi aufgefundenen grossen „Lymphoidzellen" können daher ebensowenig ein „Nebeneinander" lymphatischer und myeloider Wucherung anzeigen, wie das makroskopische Aussehen der Organe bei der Sektion oder das Verhalten der Organabstriche; beweisend könnte hierfür lediglich die histologische Untersuchung der Wucherungen, d. h. die Lokalisation der „Lymphoidzellen" im Gewebe sein.

Trotz des Protestes von Türk bleibt der von Fleischmann[1]) gemachte Einwand bestehen, dass die „Lymphoidzellen" auch „Myeloblasten" gewesen sein könnten, denn ansehen kann, unserer Meinung nach, diesen Zellen Niemand, ob sie imstande gewesen wären, sich zu echten (kleinen) Lymphozyten oder Myelozyten zu entwickeln. Der Beweis, dass Zellen vom Charakter der „grossen Lymphozyten" zu Myelozyten werden können, ist erbracht, der Beweis, dass sie identisch sind mit den Vorstufen der Lymphozyten, erscheint uns noch ausständig, sollte er geliefert werden können, so wäre die hier diskutierte Zellform (der grosse Myeloblast, die grosse Lymphoidzelle, der Grosslymphozyt), wie Pappenheim es annimmt, die Stammzelle aller Leukozyten, und es sollte ihr dann auch in der klinischen Hämatologie dieser Name gegeben werden.

Technische Bemerkungen.

(Unter Mitarbeit von W. H. Merriam.)

Vor einiger Zeit hat Schridde die Behauptung aufgestellt, dass in den Lymphozyten fuchsinophile Gebilde, die er mit den Altmannschen Granulationen identifizierte, vorkommen, dass diese aber in den ungranulierten Knochenmarkszellen nicht nachweisbar seien. Bei der prinzipiellen Bedeutung, die dieser Beobachtung zugeschrieben wurde, ist es begreiflich, dass zahlreiche Untersucher sich bestrebten, die behaupteten Tatsachen nachzuprüfen, weniger verständlich aber, dass weder Schridde selbst noch einer der Nachuntersucher Gelegenheit genommen hat, Originalpräparate einem sachkundigen und interessierten Publikum zu demonstrieren. Man war bisher auf die schematisierten Abbildungen von Schridde angewiesen.[2]) Der Grund hierfür könnte zum Teil darin zu suchen sein, dass es nicht leicht gelingt, mit der von Schridde angegebenen Modifikation der Altmannschen Methode demonstrable Präparate zu bekommen. Einzig und allein Morawitz

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[1]) Vergl. hierzu den Vortrag von Türk über „Beziehungen zwischen myeloidem und lymphoidem Gewebe", gehalten auf dem Kongress für innere Medizin in München, sowie die dazu gehörigen Diskussionsbemerkungen.
[2]) Siehe hierzu die vollkommen berechtigten Bemerkungen von Pappenheim, Fol. haemat., Bd. VII.

und Rehn[1]) bestätigten die Angaben Schriddes, indem sie schreiben: „Es gelingt leicht in Abstrichen und Schnitten von Lymphknoten und Milz die Granulationen der Lymphozyten und Lymphoblasten darzustellen. Sie finden sich meist in geringer Zahl um den Kern gelagert, sind ziemlich fein und jedenfalls mit den groben, pseudoeosinophilen Granulationen der Myelozyten, die nach Altmann-Schridde natürlich auch dargestellt werden, gar nicht zu verwechseln. In den lymphoiden Zellen des Knochenmarkes fehlen diese Granula vollständig; es sind im Knochenmark mit dieser Methode nur Granula darstellbar, die man auch auf andere Weise, z. B. durch Giemsafärbung, sichtbar machen kann."

Wenn die von Schridde eingeschlagene Modifikation der Altmannschen Methode eine brauchbare histologische Methode wäre, dann müsste sie jedem gut geschulten Histologen an geeignetem Material gelingen, und wenn die von Schridde in den Lymphozyten gefundenen .fuchsinophilen Gebilde mit den Altmannschen Granulis identisch sind, dann müssen sie sich mit denjenigen Methoden, mit denen die wichtigsten Arbeiten über die Altmannschen Granula gemacht worden sind, auch darstellen lassen. Da wir seit mehreren Jahren sehr schlechte Erfahrungen mit der Schriddeschen Modifikation der Altmannschen Methode gemacht haben, sind wir zu den ursprünglichen Methoden zur Darstellung der Altmannschen Granula zurückgekehrt. Wir haben von dem Vorschlag Marchands,[2]) die feuchte Fixationsmethode anzuwenden, in ausgiebiger Weise Gebrauch gemacht und damit sehr gute Resultate erzielt. So haben wir gleichzeitig die Schriddesche Methodik sowie die gebräuchlichsten anderen histologischen Methoden zur Fixation und Färbung der Zellstruktur angewandt. Es sind also noch feuchte Ausstriche von normalem und pathologischem Blut sowie frische.Organabstriche in Chrom·Osmiumgemischen (Altmann, Flemming, Schridde), in Sublimat (gesättigte Lösung, Sublimat-Eisessig, Zenker) mit und ohne Nachbehandlung von Osmiumtetroxyd, in Osmiumtetroxyd allein sowie lufttrockene Präparate in Methylalkohol und auf der erhitzten Kupferplatte fixiert und nach Altmann und Altmann-Schridde mit Säurefuchsin und Pikrinsäure und nach Heidenhain mit Eisenhämatoxylin gefärbt worden. Auf diese Weise glauben wir am sichersten einen Vergleich der Leistungsfähigkeit der verschiedenen Methoden erhalten zu haben. Wir geben die Resultate im folgenden wieder.

I. Osmium-Chromgemische.

a) Flemmingsche Lösung. Die noch feuchten Ausstriche werden mit der beschickten Seite nach unten in die Flemmingsche Flüssigkeit geworfen. Fixiert wird 3—6 Stunden. Die Ausstriche werden dann einzeln herausgenommen, mit Brunnenwasser gründlich abgespült und 6—12 Stunden in destilliertes Wasser gebracht (das destillierte Wasser wird häufig erneuert). Vor der Färbung wird der Ausstrich nochmals mit destilliertem Wasser abgespült und durch einfaches Abfliessenlassen von dem überschüssigen Wasser befreit.

[1]) Über einige Wechselbeziehungen der Gewebe in den blutbildenden Organen. Deutsches Archiv f. klin. Med., Bd. XCII, S. 121.
[2]) Münchner med. Woch. 1908, S. 423.

Färbung 1 nach Altmann.

1. Der Ausstrich wird mit einer dicken Schicht von Anilinwasser-Fuchsin S (20 g Fuchsin S in 100 ccm Anilinwasser) bedeckt und erhitzt, bis Dämpfe aufsteigen. Letzteres kann mit Vorteil einige Male wiederholt werden. Kalt werden lassen; die überschüssige Farblösung abfliessen lassen; Befreiung der Ränder des Präparates von etwa eingetrockneter Farblösung durch Abtupfung mit Fliesspapier.

2. Abspülung in der Altmannschen Pikrinlösung (1 Teil gesättigte alkoholische Pikrinsäurelösung zu 2 Teilen destilliertes Wasser).

3. Einlegen 40 bis 60 Sekunden in die gleiche Pikrinsäurelösung bei 50—60° (Paraffinofen).

4. Schnelle Entwässerung in absolutem Alkohol.

5. Xylol, Einlegen von Xylol ohne Abzutrocknen in Canadabalsam.

Die Bilder sind von überraschender Schönheit. Die roten Blutkörperchen sind gelbbraun, das Chromatin und die Kernkörperchen fuchsinrot, das Protoplasma der weissen Blutzellen tief gelb, und die eingelagerten neutrophilen und die Altmannschen Granula leuchtend rot, die eosinophilen schwarzrot gefärbt.

Färbung 2 nach Altmann-Schridde.

Die Modifikation von Schridde weicht nur dadurch von der ursprünglichen Altmannschen Vorschrift ab, dass eine Pikrinsäurelösung von etwas anderer Konzentration (1 Teil gesättigte alkoholische Pikrinsäurelösung auf 7 Teile 20 Proz. Alkohol) als Differenzierungsflüssigkeit ohne Erwärmung verwendet wird. Bei dieser Art der Differenzierung werden die Präparate nicht so scharf differenziert wie bei Altmann. Die roten Blutkörperchen bleiben mehr rot, die Kernstruktur der weissen Zellen wird nicht so scharf dargestellt, und die Präparate bleiben an Schönheit wesentlich hinter den Altmannschen zurück. Die Granulafärbung ist gleich wie bei Altmann.

Färbung 3 nach Heidenhain.

1. Beizung ½ Stunde in 1,5 Proz. Eisenammoniumalaun-Lösung. Abspülen mit destilliertem Wasser.

2. Färbung 2 Stunden oder länger in 0,5 Proz. wässerige Lösung von Hämatoxylin. Abspülen in Brunnenwasser.

3. Differenzierung in der gleichen Lösung von Eisenammoniumalaun wie bei 1, bis die roten Blutkörperchen nicht mehr gefärbt erscheinen. Abspülen in Brunnenwasser. (Die Dauer der Differenzierung muss ausprobiert werden; sie kann nicht in Minuten angegeben werden, da sie von der Färbekraft der Hämatoxylinlösung abhängt. Bei einiger Übung gelingt es, allerdings unter häufiger Kontrolle mittels des Mikroskopes, Präparate zu bekommen, in denen die roten Blutkörperchen vollkommen entfärbt sind, die Kerne der weissen Zellen sehr scharf blau-schwarz und das Protoplasma farblos oder sehr schwach grau tingiert ist, während die Granula in tief schwarzem Ton erscheinen.)

4. Entwässerung in absolutem Alkohol.

5. Xylol, Einlegen aus Xylol ohne Abzutrocknen in Canadabalsam.

Die Kernstruktur und die Altmannschen Granula werden sehr deutlich dargestellt; die Blutplättchen werden auch tief blauschwarz gefärbt.

b) Die Schriddesche Methode.[1]) Die Ausstriche kommen sofort nach dem Ausstreichen in

1. Formol-Müller (1 : 9) 12 Stunden.
2. Müllersche Flüssigkeit allein 12 Stunden.
3. Abspülen mit gewöhnlichem dann mit destilliertem Wasser.
4. Osmiumtetroxyd 1 Proz. Lösung 30—60 Minuten.[2])
5. Kurzes Abspülen mit destilliertem Wasser.
6. Färben in Altmannscher Anilinwasser-Fuchsin S Lösung. Die Präparate werden mit einer hohen Schicht der Farblösung beschickt und 5—6 mal über die Spiritusflamme erwärmt bis jedesmal Dämpfe abgegeben werden; erkalten lassen.

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[1]) Münchener med. Woch. 1905, S. 1233.
[2]) Hier sei es erlaubt auf einen störenden Druckfehler in dem Lehrbuch von Naegeli, „Blutkrankheiten und Blutdiagnostik", aufmerksam zu machen. Naegeli hat die Vorschrift von Schridde unrichtig wiedergegeben, da er schreibt, dass die Präparate bloss 30—60 Sekunden in Osmiumlösung fixiert werden sollen.

7. Ränder des Präparates abwischen und dann differenzieren in Pikrinsäurelösung (1 Teil gesättigte alkoholische Pikrinsäurelösung, 7 Teile 20 Proz. Alkohol) bis das Präparat einen gelblichen oder hellgelblichen Ton annimmt. Schnell in absolutem Alkohol abspülen, Xylol, Balsam.

Die Methode hat verschiedene Übelstände: 1. bei dem Fixationsverfahren löst sich die Blutschicht leicht von dem Deckglas bzw. Objektträger ab, 2. bilden sich sehr leicht störende Niederschläge infolge Anwendung der Müllerschen Flüssigkeit, 3. wird die Orientierung durch die dunkelrote Färbung der roten Blutkörperchen erschwert. Wir haben nie niederschlagsfreie Präparate bekommen können, obwohl wir möglichste Sorgfalt in die Zubereitung der Müllerschen Flüssigkeit legten. Wir haben wiederholt frische Portionen der Müllerschen mit den Kahlbaumschen Chemikalien verwendet, die Lösungen filtriert und bei der Fixation wiederholt erneuert. Es bleibt aber immer ein feiner Niederschlag über das ganze Präparat verstreut, der im ungefärbten Präparat schwach braun und im gefärbten rotbraun bis gelb erscheint. Dadurch wird die Deutung der Präparate sehr erschwert; die Lymphozytengranula werden freilich dargestellt; aber erst, nachdem wir saubere und klare Bilder mit der Flemmingschen Fixation und Altmann- bzw. Heidenhainschen Färbungsmethode gesehen haben, waren imstande, die Präparate zu deuten.

Die neue abgekürzte Methode, wie sie in dem eben erschienenen Buch von Ehrlich, Lazarus und Naegeli, „Die Anämie", S. 70, angegeben wird, hat uns ebenfalls keine besseren Resultate geliefert.

Störende Niederschläge erhält man immer, wenn man Müllersche Flüssigkeit anwendet; daher auch, wenn man die nach Schridde fixierten Präparate nach Altmann oder Heidenhain weiter behandelt.

c) Altmannsche Fixationsflüssigkeit. (Gleiche Teile von 5 Proz. Kaliumbichromat und 2 Proz. Osmiumtetroxyd.) Die Blutschicht löst sich sehr bald vom Deckglas ab; Färbung von Ausstrichen nach Altmannfixation ist deshalb nicht ausführbar.

II. Sublimatfixation mit Osmiumnachbehandlung.

a) Gesättigte Lösung von HgCl₂. Es wurde 10 Minuten, $^1/_2$ Stunde, 1 Stunde und 2 Stunden in Sublimat fixiert, gründlich gewaschen, $^1/_2$—1 Stunde mit 1 Proz. Osmiumtetroxyd nachbehandelt und schliesslich mehrere Stunden in Wasser gewaschen. Die Färbung nach Altmann, Altmann-Schridde und Heidenhain gibt bezüglich der Altmannschen Granulationen positive Bilder, wenn auch weniger schön wie bei Ia, Färbungen 1, 2 und 3.

b) Sublimateisessig. Es wurde genau wie mit der gesättigten Sublimatlösung verfahren. Resultat in allen Einzelheiten das gleiche.

c) Zenkersche Flüssigkeit (mit Zusatz von Eisessig). Hier wurde wiederum 10 Minuten, $^1/_2$ Stunde, 1 Stunde, 2 und 6 Stunden fixiert. Nachbehandlung mit Osmiumtetroxyd 1 Stunde, gründliches Auswaschen. Färbung nach Altmann, Altmann-Schridde und Heidenhain. Die Präparate sind viel instruktiver als bei alleiniger Sublimatfixation. Nach der Fixation mit Zenkerscher Lösung stellen sich bei Färbung nach Altmann und Altmann-Schridde die Lymphozytengranula in ihrer Form etwas anders dar, als bei der Fixation nach Flemming oder Schridde. Sie haben eine mehr länglich ovale Form. Sie lassen sich zwar auch mit Eisenhämatoxylin darstellen, das Resultat ist aber ein viel weniger klares.

IIa. Sublimatfixation ohne Osmium.

Es wurde wie bei IIa, b und c mit Weglassen der Nachbehandlung mit Osmiumtetroxyd verfahren. Gefärbt wurde nach Altmann, Altmann-Schridde und nach Heidenhain. In keinem Fall gelang es in den Lymphozyten Granula aufzufinden; das Protoplasma erscheint dagegen völlig homogen. Das Vorhandensein von Osmiumtetroxyd bei dem Fixationsprozess scheint deshalb unbedingt erforderlich zu sein.

III. Osmiumtetroxyd allein.

Fixation in 1 Proz. Lösung von Osmiumtetroxyd 2 Stunden, gründliches Auswaschen, Färbung nach Altmann. Durch diese höchst einfache Methodik werden die Lymphozytengranula sehr scharf dargestellt. Die Methode ist aber nicht besonders zu empfehlen, da die Kernstruktur lange nicht so schön dargestellt wird wie bei der Fixation nach Flemming.

IV. Methylalkohol und Hitzefixation der lufttrockenen Präparate.

Durch Färbung nach Altmann, Altmann-Schridde und Heidenhain werden keine Granulationen in den Lymphozyten nachweisbar gemacht.

Aus diesen vergleichend-technischen Untersuchungen ergibt sich, dass die sicherste Methode zur Darstellung der Altmannschen Granula in Blutzellen die feuchte Fixation in Flemmingscher Lösung und die Färbung nach Altmann ist; doch können die Granula nach jeder Methode, die Osmiumtetroxyd enthält, dargestellt werden. Unser Verfahren (s. Ia und Färbung 1) ist ausserordentlich viel einfacher als die Modifikation von Schridde, das Resultat ein bedeutend sicheres, und die Beurteilung der Präparate eine leichtere.

Nachdem wir in der Darstellung der Lymphozytengranula die notwendige Sicherheit erlangt hatten, konnten wir uns an die Aufgabe heranwagen, zu untersuchen, welche Zellen der beiden Leukozytenreihen die genannten Granula aufweisen. In der normalen Histologie und bei typischen Fällen von Leukämie wären Unterschiede, die eine Trennung der Lymphozyten und Markzellen von einander gestatten, von nicht so prinzipieller Bedeutung. Wohl aber für Fälle von akut verlaufender Leukämie mit vielen grossen ungranulierten Zellen im Blut. Hier wäre es sehr erwünscht, wenn man von einer einzelnen Zelle bestimmt sagen könnte, „diese stammt aus lymphatischem Gewebe", oder „diese Zelle ist eine ungranulierte Vorstufe der Myelozyten". Wir haben nun bereits in einer früheren Arbeit gerade an solchen Fällen mit einem Überwiegen von grossen ungranulierten Zellen bis zu 90 Proz. im Blut, die Kriterien von Naegeli und Schridde auf die Probe gestellt und gefunden, dass bei Fällen von myeloider Leukämie (mit Sektion) fast alle die von Schridde und Naegeli als charakteristisch für die „Lymphoblasten" beanspruchten morphologischen Eigenschaften den ungranulierten Vorstufen der Myelozyten auch zukommen. Das einzige was uns früher fehlte, war der Nachweis der perinukleären, fuchsinophilen Gebilde in den bei gewöhnlichen Färbungen ungranulierten myeloiden Zellen. Ihr Nachweis war uns wegen der Schwierigkeit der Methode wiederholt missglückt; es gelang uns nicht einmal in den Lymphozyten immer klare und überzeugende Bilder zu bekommen.

In einem neuen Fall haben wir mit den in den vorangehenden Zeilen beschriebenen Methoden systematisch intra vitam und post mortem untersucht, und nun auch den noch fehlenden Nachweis in den „Myeloblasten" geliefert. Der Fall war folgender:

55jährige Frau, hochgradig anämisch, Venenpulsation am Halse, systolische Geräusche am Herzen, Sternum auf Beklopfen druckempfindlich, Leber vergrössert, palpabel, unterer Rand fingerbreit über den Nabel; Milz 13 cm, hart, palpabel; leichtes Ödem am Kreuz und an den Füssen. Die Kranke war 8 Tage lang in klinischer Beobachtung, hatte in den 3 ersten Tagen Temperaturen zwischen 39 und 40, in den folgenden Tagen zwischen 38 und 39°. Der Puls bewegte sich zwischen 100 und 125, die Respiration zwischen 30 und 40. Zweimal angelegte Blutkulturen blieben steril. Wassermannsche Reaktion negativ. Keine Blutungen im Augenhintergrund.

Keine Vermehrung von Hämatoporphyrin und Urobilin im Harn. Hohe Harnazidität, viel freie Harnsäure. Unter allgemeiner Schwäche und heftigen Delirien Exitus.

Blutbefund: Erythrozyten 1 300 000 im Kubikmillimeter, Hämoglobin (20 Proz.) (Sahli), weisse Blutkörperchen 27,000 im Kubikmillimeter. Die Auszählung des Jenner-May Präparates ergab:

46,9 Proz. polymorphkernige Neutrophile,
19,1 einkernige Neutrophile,
26,2 Proz. grosse einkernige ungranulierte Zellen,
5,1 Proz. kleine Lymphozyten,
1,4 Proz. Eosinophile,
1,3 Proz. Mastzellen.

Auf 640 weisse Blutzellen 3 Normoblasten. Das Blutbild war beherrscht von myeloiden Zellen, von denen die ungranulierten alle Kriterien der Vorstufen und alle Übergänge zu den neutrophilen Myelozyten aufwiesen. Auf ihre genaue Beschreibung können wir hier verzichten, da sie sich in allen Eigenschaften mit den in der Arbeit über die ungranulierten Vorstufen der Myelozyten auf Tafel VII und VIII abgebildeten Zellen vollständig deckten. Nebenbei sei bemerkt, dass viele dieser sicheren Myeloblasten bei allen Färbungsmethoden nur ein Kernkörperchen aufweisen.

Die Sektion ergab den Befund einer mycloiden Leukämie mit leicht hyperplastischem Knochemark und bedeutendem Milztumor, ohne Lymphdrüsenvergrösserung.

Die histologische Untersuchung ergab myeloide Umwandlung der Milz, leukämische Hyperplasie des Knochenmarks mit sehr zahlreichen grossen ungranulierten Zellen, von denen alle Übergänge zu den Myelozyten vorhanden waren.

Das Resultat der Untersuchung auf Altmannsche Granulationen wird ohne viel Worte am einfachsten durch unsere Abbildungen illustriert.

Um unsere Schlussfolgerungen nicht allein auf pathologisches Material stützen zu müssen, haben wir normales Blut, Blut von Lymphämien und normales Knochenmark nach den gleichen Methoden gefärbt und in unseren Abbildungen wiedergegeben.

Durch die Untersuchung ist der Beweis erbracht, dass auch die ungranulierten Vorstufen der Myelozyten (Myeloblasten) perinukleäre, fuchsinophile Gebilde (Altmannsche Granulationen) enthalten, die nicht von denen der Lymphozyten zu unterscheiden sind.

——— —

Zusatz bei der Korrektur:

Nach Abschluss dieser Mitteilung konnten wir zwei Fälle beobachten, bei denen das Blutbild der typischen chronischen myeloiden Leukämie sich während der Beobachtung derart umwandelte, dass in dem einen Fall 50 Prozent, in dem anderen 90 Prozent der grossen ungranulierten Zellen (sogenannte grosse Lymphozyten) vorhanden waren. Auch hier wurden in den grossen fraglichen Zellen, die sich bei allen anderen Färbungen als ungranuliert erwiesen, die Altmannschen Granula perinukleär sowohl im Blut, wie in den myeloid umgewandelten Organen nachgewiesen.

Erklärung der Abbildungen.

Zur Illustration des Gesagten geben wir einige möglichst naturgetreue von Herrn Kunstmaler Dirr ausgezeichnet reproduzierte Zellabbildungen wieder:

Die mit arabischen Zahlen 1—9 bezeichneten Zellen sind sichere Lymphozyten.

    1 und 2 aus normalem Blut,
    3 bis 6 von Fällen mit Lymphozytose,
    7 bis 9 aus lymphatischer Leukämie.

Die mit römischen Zahlen bezeichneten Zellen stammten alle aus dem beschriebenen Fall akuter myeloider Leukämie.

I, II und III sind Zellen, die im Eosin-methylenblau und im Giemsa-Präparat intensiv basophiles, scholliges Protoplasma häufig mit zahlreichen Vakuolen besitzen, und denen mit den gewöhnlichen Blutfärbungsmethoden (auch mit Triazid) alle Granulationen fehlen. Von ihnen sind alle Übergänge zu den Myelozyten vorhanden.

IV, V, VI und VII sind Zellen desselben Falles mit perinukleären fuchsinophilen Granulationen, die höchst wahrscheinlich das Bindungsglied der Zellen I bis III zu den echten Myelozyten darstellen.

Die Zelle VIII würde wohl der Kernstruktur und dem allgemeinen Typus nach als Lymphozyt gelten können; sie demonstriert am deutlichsten wie schwer es ist, einer derartigen Zelle selbst bei Anwendung aller zur Verfügung stehenden Methoden, eine bestimmte Zugehörigkeit zu dem einen oder anderen Zellsystem zuzuschreiben. (Leider ist dies in der Reproduktion nicht recht zu erkennen.)

Die Färbung sämtlicher Zellen mit Ausnahme von 2, 6, 8 und 9 ist die oben angegebene Altmannsche nach Fixation mit Flemmingscher Lösung.

2, 6, 8 und 9 sind auch in Flemmingscher Lösung fixiert und nach Heidenhain mit Eisenhämatoxylin gefärbt.

Die Zelle II zeigt, dass die in Eosin-Methylenblau-Präparat gesehenen Protoplasmavakuolen eine fettartige, mit Osmiumtetroxyd sich schwärzende Substanz enthalten.
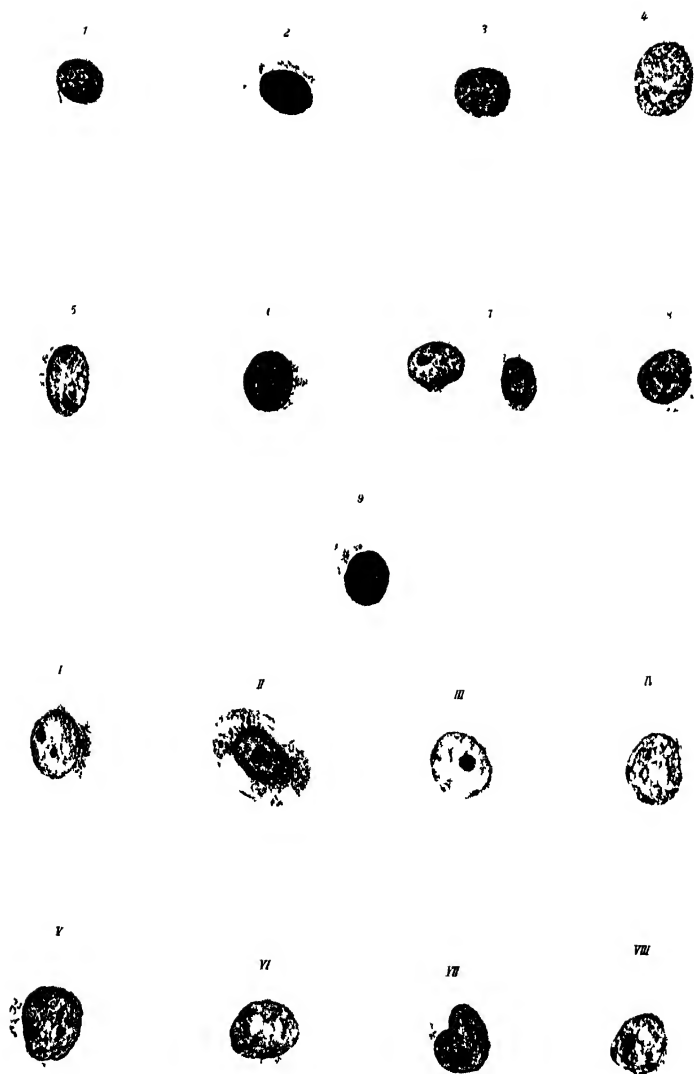
[Aus dem Königl. Institut für experimentelle Therapie zu Frankfurt a. M. (Direktor: Geh. Obermed.-Rat Prof. Dr. P. Ehrlich).]

Ueber einen arsenfesten Bakterienstamm.

Von Dr. **Lewis H. Marks** [1],
Assistenten am Institut.

Die grundlegenden Arbeiten E h r l i c h s , welche die Festigkeit der Trypanosomenstämme gegenüber verschiedenen Chemikalien im Tierkörper behandeln, haben neues Interesse auf diesem biologisch so wichtigen Gebiete geweckt und haben die Anregung zu folgenden Studien gegeben.

Während der letzten 3 Jahre haben wir in dieser Richtung mit Bakterien und Hefe im Reagenzglas gearbeitet und haben versucht, die Veränderungen nicht allein der Chemozeptoren, sondern auch die Eigentümlichkeiten dieser Bakterien im Verlaufe der Gewöhnung an gewisse Chemikalien zu studieren.

Wir wollen in dieser vorläufigen Mitteilung eine kurze Uebersicht über unsere Resultate geben, die wir mit einem gewissen Bakterienstamm — einem Paratyphusstamm aus der Hog-Choleragruppe —, der einer längeren Einwirkung von arseniger Säure ausgesetzt war, erzielt haben. Anpassung von Bakterien an Arsenpräparate ist bekanntlich schon früher (D a n y s z , R u p p e l u. a.) beschrieben worden.

Die von uns benutzte Kultur stammt von einem Fall von Fischvergiftung und war schon lange Zeit im hiesigen Laboratorium fortgezüchtet. Als Nährboden kam aus technischen Gründen nur Agar zur Verwendung.

Wir haben zuerst versucht, zu ermitteln, ob die arsenige Säure die gleiche Wirkung auf alle einzelnen Individuen der Kultur ausübt, und haben zu diesem Zwecke von einem Endo-Agarsatz drei Kolonien isoliert und jedesmal eine Kolonie auf Endo-Agar ausgestrichen. Von den letzten drei Endo-Agarsätzen nahmen wir je eine isolierte Kolonie zum Versuch. Es seien die Kolonien A, B, C. Das Verhalten der drei Kolonien gegen arsenige Säure zeigt die folgende Tabelle.

1

Tabelle.

Arsenige Säure verdünnt in gewöhnlichem Agar	Verschiedene Individuen vom Ausgangsstamm		
	A	B	C
1 : 34 000	gewachsen	gewachsen	gewachsen
1 : 33 000	„	„	„
1 : 32 000	„	„	„
1 : 31 000	kein Wachstum	„	„
1 : 30 000	„ „	„	„
1 : 29 000	„ „	„	kein Wachstum
1 : 28 000	„ „	„	„ „
1 : 27 000	„ „	„	„ „
1 : 26 000	„ „	kein Wachstum	„ „
1 : 25 000	„ „	„ „	„ „
1 : 24 000	„ „	„ „	„ „
Kontrollen	gewachsen	gewachsen	gewachsen

Es geht also aus der Tabelle hervor, daß die Abkömmlinge ein und derselben Kultur ein verschiedenes Verhalten gegenüber der arsenigen Säure zeigen. Dieses Resultat stimmt mit den von Eisenberg mitgeteilten Beobachtungen über verschiedene Agglutinierbarkeit der einzelnen Individuen derselben Kultur überein.

Es wurde dann versucht, die Bakterien gegen das Mittel fest zu machen, und zu diesem Zweck wurden mehrere Reihen absteigender Mengen von arseniger Säure mit Agar gemischt.

Wir haben zu diesem Versuch die Kultur B (s. Tabelle) benutzt, die, wie ersichtlich, noch bei einem Zusatz von 1 : 27 000 arseniger Säure deutliches Wachstum zeigte. Unter langsamer allmählicher Steigerung der Konzentration haben wir nach 3 Jahren mühevoller Arbeit erreicht, daß die Kultur jetzt auf Agar wächst, dessen Konzentration an arseniger Säure 1 : 3500 beträgt, also etwa 8-fach stärker ist, als die dem Ausgangsstamm ein Wachstum noch ermöglichende maximale Konzentration.

Als der Vorrat an Agarröhrchen mit den einzelnen Verdünnungen arseniger Säure erschöpft war und eine neue Serie von Agarverdünnungen angefertigt werden mußte, ergab sich die interessante Beobachtung, daß die Kultur bei der Konzentration, die eben noch in dem alten Röhrchen ein Wachstum zuließ, schon im Wachstum unterdrückt wurde, doch konnte sehr schnell die frühere Toleranz gegen das Mittel erreicht werden.

Was die Technik der Höhertreibung der Resistenz anbetrifft, so empfahl es sich, langsam vorzugehen

und die Kulturen mehrmals auf der gleichen Konzentration fortzuführen, damit sie sich vollkommen an dieselbe adaptieren.

Die Steigerung der Festigkeit geht übrigens nicht gleichmäßig progressiv vor sich, sondern erfolgt etappenweise. So kann es vorkommen, daß bei einer bestimmten erreichten Höhe monatelang auf derselben Konzentration fortgezüchtet werden muß, ehe man die Konzentration steigern kann. Solche Stufen konnten wir insbesondere bei den Konzentrationen von 1:15000, 1:12000, 1:8000 beobachten.

Veränderungen der allgemeinen Eigentümlichkeit der Bakterien.

1) Die Kultur wird sofort unter dem Einfluß der arsenigen Säure unbeweglich. Die Unbeweglichkeit besteht so lange, als die Kultur auf arsenhaltigem Nährboden gezüchtet wird, letztere gewinnt aber nach einigen Passagen auf gewöhnlichem Agar die Beweglichkeit wieder.

2) Die Geißelfärbung zeigt eine viel geringere Zahl und Verkürzung der einzelnen Geißeln als beim Ausgangsstamm.

3) Die Bakterien erscheinen im Ausstrichpräparat kürzer und plumper und neigen zu längerer Kettenbildung, die den Eindruck hervorruft, als ob es sich um ein einziges, sehr langes Bakterium handelte.

4) Auf gewöhnlichem Agar und Löffler-Serum zeigt das Wachstum, abgesehen von einer Verminderung, keine Besonderheiten.

5) Ein sehr eigentümliches Wachstum zeigen diese arsenfesten Bakterien auf Endo-Agar. Bei einer Toleranz von 1:15000 arseniger Säure sind die Ränder der Kolonien sehr unregelmäßig und ausgefranst. Ihre Farbe ist verschieden, teils sind die Kolonien ganz weiß, teils sind sie rot mit einer weißen Zentralzone, teils ganz rot. Bringt man eine rote Kolonie durch Abstechen wieder auf Endo-Agar, so wachsen darauf sowohl weiße als auch die oben beschriebenen rotweißen Kolonien. Durch mehrmalige Wiederholung der Prozedur, wobei man jedesmal eine rotweiße Kolonie absticht, erhält man endlich nur noch ganz weiße Kolonien, wie die Originalkultur. Die Zahl der erforderlichen Ueberimpfungen hängt von der Höhe der Toleranz ab, je stärker die Arsen-

festigkeit ist, desto mehr Passagen sind erforderlich, damit die Bakterien ihr normales Wachstum wieder zeigen.

Bei der maximalen Festigkeit (1:3500) ist das Wachstum auf Endo-Agar sehr erheblich gehemmt. Bis zum 3. Tage nach der Ueberimpfung sind die Kolonien nur mit der Lupe zu sehen, sind alle rot und zeigen einen typischen „Coliglanz".

Veränderungen der biochemischen Eigenschaften.

Originalstamm vergärt Traubenzucker am 1. Tag, und Lackmusmolke wird tiefblau.

Arsenstamm 1:20000 fest: Traubenzucker vergoren am 2. Tag, Lackmusmolke blau am 7. Tag.

Arsenstamm 1:15000 fest: Traubenzucker nicht vergoren, Lackmusmolke unverändert. Sonst keine Besonderheiten.

Haltbarkeit der Veränderung.

Ein Arsenstamm von der Festigkeit 1:8000 brauchte 46 Passagen auf gewöhnlichem Agar, um wieder das normale Kulturverfahren zu geben. Wir beobachten jetzt einen Stamm von 1:4000 Festigkeit, den wir 90mal auf gewöhnlichen Agar überimpften, ohne normale Kulturverhältnisse wieder erreicht zu haben. Es ist möglich, daß dieser Stamm noch längere Zeit überimpft werden muß, bevor er die kulturellen Eigenschaften des Ausgangsstamms wieder erlangt. Es wäre aber auch denkbar, daß er ein Stadium erreicht hat, wo eine Rückkehr zum kulturellen Verhalten der Ausgangskultur nicht mehr möglich ist. Ganz ähnliche Verhältnisse haben wir schon in der Beobachtung Ehrlichs, der von einem atoxylfesten Trypanosomenstamm Mitteilung macht, der trotz dreijähriger Passage durch normale Mäuse seine Festigkeit gegen Atoxyl nicht eingebüßt hat.

Veränderungen der Agglutinierbarkeit.

Oft wiederholte Versuche gaben für den Originalstamm einen Titer von 1:104000 mit einem durch Vorbehandlung eines Kaninchens mit dem Originalstamm gewonnenen Immunserum. Ein polyvalentes Schweinepestserum agglutiniert noch in einer Verdünnung 1:50000, ein Paratyphus-B-Serum in der Verdünnung 1:100.

Die Agglutinierbarkeit des festen Stammes (1:14000) gegenüber den angeführten Seren war:

1) Immunserum des Ausgangsstammes, Verdünnung 1 : 12 000.

2) Schweinepestserum 1 : 16 000.

3) Paratyphus-B-Serum 1 : 400.

Von besonderem Interesse ist der Umstand, daß, wie ersichtlich, die Agglutinierbarkeit gegenüber dem homologen Immunserum, sowie dem Schweinepestserum eine erhebliche Verminderung erfahren hatte, während diejenige gegenüber dem Paratyphus-B-Serum sich deutlich gesteigert erwies. Noch deutlicher war der Unterschied bei einer Festigkeit von 1 : 6000:

1) Immunserum des Ausgangsstammes: 1 : 6400.

2) Schweinepestserum: 1 : 100.

3) Paratyphus-B-Serum: 1 : 800.

Ein Serum, das durch Vorbehandlung mit einem (1 : 6000) festen Stamm gewonnen wurde, agglutinierte den homologen Stamm und die Originalausgangskultur in einer Verdünnung von 1 : 12 800.

Eine bis jetzt noch nicht aufgeklärte Eigentümlichkeit, die jedoch durch mehrmalige Nachprüfung sichergestellt wurde, zeigte das eben erwähnte Immunserum gegenüber einem Stamm, der früher 1 : 8000 fest war, durch wiederholte Passagen auf gewöhnlichem Agar aber sein normales kulturelles Verhalten wiedererlangt hatte, indem es diesen Stamm in einer Verdünnung von 1 : 108 400 noch deutlich agglutinierte.

In einer nächsten Publikation sollen weitere agglutinative Veränderungen und das Resultat der Komplementbindungsversuche Erwähnung finden.

Es braucht kaum hervorgehoben zu werden, daß alle oben erwähnten kulturellen und Agglutinationsversuche mehrmals mit stets dem gleichen Resultat ausgeführt wurden. Die Agglutinationsversuche wurden außerdem noch getrennt im städtischen hygienischen Institut (Dir.: Prof. Dr. Neisser) durch Frl. Krüger kontrolliert, denen ich an dieser Stelle meinen besten Dank für die Liebenswürdigkeit und Mühe aussprechen möchte.

Veränderung der Chemozeptoren.

Genau wie Ehrlich gezeigt hat, daß atoxylfeste Trypanosomen gegen Arsenderivate und Antimon fest sind, verhält

sich auch unser arsenfester Bakterienstamm. Auf Antimon-agar wächst der Ausgangsstamm in einer Konzentration von 1 : 12 000, der arsenfeste Stamm dagegen in einer Konzentration von 1 : 250. Die Resistenz gegenüber Antimon ist also um das 40-fache gestiegen, während diejenige gegenüber arseniger Säure sich nur um das 8-fache vermehrt hatte. D e r U m s t a n d , d a ß d u r c h d i e B e h a n d l u n g m i t a r s e n i g e r S ä u r e d i e F e s t i g k e i t g e g e n ü b e r A n t i m o n i n e i n e m 5 - f a c h h ö h e r e m M a ß e g e s t i e g e n w a r , a l s g e g e n- ü b e r d e m z u r B e h a n d l u n g d i e n e n d e n M i t t e l , d ü r f t e b e s o n d e r s b e m e r k e n s w e r t s e i n.

Ferner darf vielleicht an dieser Stelle darauf hingewiesen werden, daß es E h r l i c h nicht gelungen ist, bei Trypanosomen eine Festigkeit gegen arsenige Säure zu erzielen, und der Gegensatz, in dem unsere Bakterienversuche dazu stehen, dürfte vielleicht darin eine Erklärung finden, daß hier die interferierende Rolle des Tierkörpers bei der Gewöhnung an arsenige Säure fortfällt.

Gegen Reduktionsprodukte von Atoxylderivaten — Arseno-phenylglycin und Phenolarsenoxyd — verhalten sich der Ausgangsstamm und der arsenfeste Stamm wie folgt:

Wachstum des Ausgangsstammes
    a) bei Arsenophenylglycin· 1 : 9000,
    b) bei Phenolarsenoxyd 1 : 11 000.
Wachstum des festen Stammes
    a) bei Arsenophenylglycin 1 : 3000,
    b) bei Phenolarsenoxyd 1 : 4000.

Auch gegenüber den aromatischen Arsenverbindungen war also eine immerhin 3-fache Steigerung der Resistenz wahrzunehmen.

Zusammenfassung.

Es wird über einen Stamm von Hog-cholera-Bakterien berichtet, der gegenüber arseniger Säure durch systematische Gewöhnung eine hochgradige Festigkeit erworben hatte. Von besonderem Interesse sind dabei die Veränderungen im kulturellen Verhalten, in der Agglutinierbarkeit, sowie der Umstand, daß der arsenfeste Stamm sich gleichzeitig gegenüber Antimon fest erwies.

Über die Todesursache nach intravenöser Injection von artfremdem Blutserum.[1])

(Aus dem Laboratorium für experimentelle Pathologie der University of Pennsylvania.)

Von

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Im Verlaufe von Untersuchungen über Thrombose und Blutgerinnung schien es uns von Interesse, die Wirkung einer intravenösen Injektion von artfremdem

[1]) Die Mittel zur Ausführung dieser Versuche wurden uns von dem Rockefeller Institute for Medical Research zur Verfügung gestellt.

Blutserum, und insbesondere die Bedingungen, unter denen eine solche Injektion den Tod des Versuchstieres herbeiführt, näher zu analysieren. Soweit uns bekannt, liegen über diese Frage nur wenige Mitteilungen vor. Im wesentlichen handelt es sich hierbei um die Untersuchungen von L a n d o i s [1]) und P o n f i c k [2]).

L a n d o i s fand, daß Hundeserum im Reagenzglas ein Zusammenkleben der Erythrozyten des Kaninchenblutes bewirkte; durch Ausziehen der agglutinierten Zellmassen bilde sich Stromafibrin. Gleichzeitig beobachtete L a n d o i s ein Austreten des Hämoglobins aus den veränderten Blutkörperchen. Andere Sera, wie Lammserum, Rinderserum usw. erwiesen sich als weniger stark hämolytisch für Kaninchenblutzellen.

So erklärte denn dieser Forscher den Tod nach intravenöser Injektion von artfremdem Serum als verursacht durch die Verstopfung kleiner Lungengefäße durch solche Pfröpfe von Stromafibrin, an die sich dann weiterhin eine Ausscheidung von plasmatischem Fibrin anschließen soll. Diese Annahme war geeignet, die dem Tode vorangehende Dyspnoe sowie die bei der Autopsie gefundenen Infarkte, Ödeme und Hämorrhagien in der Lunge zu erklären.

Hier muß nun bemerkt werden, daß schon vor der Veröffentlichung von L a n d o i s N a u n y n [3]) und F r a n c k e n gefunden hatten, daß Mittel, welche die Erythrozyten nach intravenöser Injektion zerstören, zur Thrombenbildung mit Fibrinausscheidung führen.

Die Ergebnisse von L a n d o i s fanden jedoch nicht allgemeine Anerkennung. Insbesondere fand P o n f i c k, daß Hunde große Mengen von artfremdem Serum vertragen konnten. Nach Injektion von Blut trat der Tod ein hauptsächlich als eine Folge von Nierenläsionen. Koagula, die sich in den Gefäßen fanden, bildeten sich infolge der Einführung der Injektionsnadel, oder waren erst postmortal entstanden. Also im wesentlichen bestritt P o n f i c k die Richtigkeit der von L a n d o i s gegebenen Erklärungen. Der Widerspruch in den Ergebnissen dieser beiden Autoren findet zum Teil darin seine Erklärung, daß P o n f i c k hauptsächlich mit Lammblut arbeitete, während L a n d o i s insbesondere die Wirkung von Hundeserum untersuchte. Als Versuchstiere benutzte L a n d o i s Kaninchen oder Hunde.

Wir beschränkten uns in den folgenden Versuchen auf die Prüfung zweier Sera, und zwar wählten wir Hunde- und Rinderserum. Als Versuchstier diente uns ausschließlich das Kaninchen. Wir zogen es vor, die Wirkung einiger weniger Sera auf eine einzige Tierart eingehend zu analysieren, anstatt eine größere Anzahl von Sera in einer im einzelnen geringeren Zahl von Versuchen zu prüfen.

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[1]) L a n d o i s , Die Transfusion des Blutes. Leipzig 1875.
[2]) P o n f i c k , Experimentelle Beiträge zur Lehre von der Transfusion. Virch. Arch. Bd. 62, 1875.
[3]) N a u n y n , Untersuchungen über Blutgerinnung im lebenden Tiere und ihre Folgen. Arch. f. exper. Path. u. Pharm. 1873 Bd. 1.

I. Versuche mit Hundeserum.
1. Injektion von auf Eis gehaltenem, möglichst unverändertem Hundeserum.

Zu diesen Versuchen dienten 63 Kaninchen. Das Blutserum von ungefähr 20 Hunden wurde in den verschiedenen Experimenten benutzt. Wesentliche Unterschiede zwischen den Blutsera fanden sich nicht. Nach stattgefundener Koagulation wurde das Blut im Eisschrank gehalten; das Serum wurde vor dem Gebrauch zentrifugiert. Während manche Sera hämoglobinfrei waren, enthielten andere mehr oder weniger gelöstes Hämoglobin. Falls die Hunde kürzere Zeit vor der Blutentnahme gefüttert worden waren, enthielt das Serum emulgiertes Fett. In der Mehrzahl der Versuche fasteten die Tiere 20 Stunden vor der Blutentnahme. Fett- oder Hämoglobingehalt der Sera schien auf die Wirksamkeit der Sera keinen stärkeren Einfluß auszuüben. Zur Injektion wurde eine Spritze benutzt; in den meisten Versuchen geschah die Einspritzung durch die Ohrvene des Kaninchens; doch wurde in einigen Fällen die Vena jugularis externa hierzu verwendet. Die Einflußgeschwindigkeit betrug in der Mehrzahl der Versuche 4 ccm pro Minute; in einer größeren Zahl von Experimenten wurde eine langsamere Injektion (2 ccm pro Minute) in Anwendung gebracht. Auch in diesem Falle ergaben sich keine wesentlichen Unterschiede, die auf Verschiedenheit der Einflußgeschwindigkeit zurückgeführt werden konnten. Bei der Feststellung der Wirkung der Sera ist es nötig, das Gewicht der Kaninchen zu berücksichtigen, da ein größeres Tier auch eine größere Quantität Blutserums vertragen kann. Die im folgenden angegebenen Quantitäten injizierten Blutserums sind daher alle auf 1000 g Kaninchen reduziert.

Letale Dose des Hundeserums. Die minimale Dose, die innerhalb der ersten zehn Minuten nach Beendigung der Injektion den Tod herbeiführt, liegt ungefähr zwischen 7 bis 10 ccm pro Kilogramm Kaninchen. Verschiedene Sera sind nicht gleich stark; aber falls das Serum in der oben angegebenen Weise behandelt wird, sind die Abweichungen nicht sehr bedeutend. Zuweilen findet man Sera, von denen 5 bis 6 ccm pro Kilogramm Tier innerhalb der ersten 10 Minuten töten.

Injiziert man verschiedene Quantitäten desselben Serums in mehrere Kaninchen, so findet man, daß Erhöhung der Dose den Tod beschleunigt; doch werden zuweilen kleine Unregelmäßigkeiten beobachtet.

Trägt man die Quantitäten des injizierten Serums auf eine Abszisse und die Anzahl Minuten bis zum Eintritt des Todes auf die Ordinate auf, so enthält man eine charakteristische Kurve, die zeigt, wie mit Zunahme des Serums die Zeit bis zum Eintritt des Todes erst sehr stark abnimmt und dann bei Zufügen von mehr Serum die Verkürzung dieser Periode immer geringer wird.

Einige Beispiele mögen angeführt werden, um die Variationen in dem Verhalten verschiedener Sera zu zeigen.

S e r u m 16 (20 bis 24 Stunden alt). 7 ccm: Tod nach 6 Minuten; 7,2 ccm: Tod nach 6 Minuten; 7,8 ccm: Tod nach 2 Minuten; 8,6 ccm: Tod nach 3½ Minuten.

S e r u m 15. In drei Tieren, in denen je 10 ccm injiziert wurden, variiert die Zeit bis zum Eintritt des Todes zwischen 4 und 5 Minuten.

S e r u m 14. 8 ccm: Tier bleibt am Leben; 11½ ccm: Tod nach 2 Minuten.

S e r u m 13. 6,6 ccm: Tod nach 23 Minuten; 9,3 ccm: Tod nach 2 Minuten.

S e r u m 12. 8,3 ccm: Tod nach 34 Minuten; 10 ccm: Tod nach 3 Minuten; 11 ccm: Tod nach 2 Minuten.

S e r u m 3. 5 ccm: Tod nach 7 Minuten; 5 ccm: Tier bleibt am Leben; 9 ccm: Tier stirbt in 1½ Minute.

S e r u m 2 (8 Tage alt). 9,2 ccm: bleibt am Leben; 10 ccm: Tod nach 62 Minuten; 12 ccm: Tod nach 7 Minuten; 14 ccm: Tod nach 4 Minuten.

Serum 3 ist ein sehr stark, Serum 2 ein relativ schwach wirkendes Serum.

S y m p t o m e. Wird eine große Quantität von Serum injiziert, so stirbt das Tier entweder am Ende der Injektion oder während der Injektion unter Konvulsionen.

Tritt der Tod innerhalb der ersten 10 Minuten ein, so findet sich oft direkt nach Beendigung der Einspritzung eine kurze Latenzperiode, während der das Tier anscheinend wohl ist; dann beobachtet man Beschleunigung der Respiration, das Tier wird schwach; darauf nimmt die Schwäche und Dyspnoe zu, zugleich wird das Tier unruhig, sodann folgen Konvulsionen und Tod.

Tritt der Tod später ein, so findet sich erst eine Periode beschleunigter Respiration und Schwäche, dann erholt sich das Tier, um dann später wieder ähnliche Symptome zu zeigen und unter Konvulsionen zu sterben.

Doch können auch zuweilen die Konvulsionen ausbleiben; und in wenigen Fällen wurden keine Konvulsionen beobachtet, obwohl das Tier innerhalb der ersten 10 Minuten nach der Injektion starb.

Die zeitliche Aufeinanderfolge der Symptome ergibt sich aus folgenden Daten:

In einem Fall, in dem der Tod nach 9 Minuten eintrat, war 2½ Minuten nach Beendigung der Injektion die Atmung beschleunigt, nach 3 Minuten war Schwäche bemerkbar, nach 6 bis 8 Minuten wurde das Tier unruhig, die Schwäche nahm zu, die Dyspnoe war beträchtlich. Nach 8 Minuten traten Konvulsionen ein und eine Minute später starb das Tier.

In einem andern Fall, in dem der Tod nach 6 Minuten eintrat, war nach 2 Minuten die Respiration beschleunigt und erschwert, nach 3 Minuten war das Tier schwach, nach 4 Minuten Zunahme der Schwäche und Dyspnoe, nach 5 Minuten Konvulsionen und nach 6 Minuten Tod.

B e f u n d e b e i d e r A u t o p s i e. Bei der sofort nach eingetretenem Tode vorgenommenen Autopsie finden sich zuweilen gar keine Veränderungen, falls der Tod spät eintrat; in der großen Mehrzahl der Versuche finden sich aber Veränderungen im Herzen und in den Gefäßen und in den Lungen. Im Herzen und in den Venen können sich Blutkoagula finden. Außerdem ist die rechte Seite des Herzens häufig erweitert. Die Blutkoagula bilden sich zuerst im rechten Herzen, können sich sodann auch in die Hohlvenen und die Portalvene fortsetzen

und, falls der Tod innerhalb der ersten Minuten nach Beendigung der Injektion eintrat, finden sich zuweilen auch Koagula im linken Herzen.

Aber diese Koagula finden sich nur, falls der Tod innerhalb der ersten 9 Minuten eintrat. Sie finden sich regelmäßig, wenn das Tier in den ersten 6 Minuten stirbt, sie sind oft, aber nicht immer vorhanden, falls der Tod zwischen der 6. und 9. Minute erfolgt, und sie sind regelmäßig abwesend, falls der Tod später als 9 Minuten nach Beendigung der Injektion stattfindet. Nur in einem Falle, in dem der Tod später eintrat, fand sich als eine Ausnahme ein kleines Gerinnsel im rechten Herzen vor.

Sind nun diese Herz- und Venenthromben die Ursache des Todes? Es läßt sich nachweisen, daß dies nicht der Fall ist. In mehreren Versuchen wurden das Herz und die Venen einiger Kaninchen bei gerade beginnender Agonie untersucht, und hier fehlten die Herz- und Venenkoagula, und erst diejenigen Tiere, die am Ende der Agonie in derselben Versuchsserie untersucht wurden, wiesen solche Thrombenbildung auf. Diese Herz- und Venenthromben bilden sich daher erst, nachdem die zum Tode führenden Veränderungen bereits eingesetzt haben; sie sind nicht verantwortlich für den Tod; es handelt sich hier um agonale Thromben.

Ähnliches gilt wahrscheinlich für die in den größeren Lungengefäßen häufig auftretenden Koagula, soweit sie makroskopisch erkennbar sind. Solche Koagula finden sich häufig, aber nicht immer, und sowohl bei Tieren, die innerhalb der ersten 10 Minuten nach der Injektion, wie auch bei solchen, die später starben. Aber auch hier konnten wir beobachten, daß anscheinend die Bildung dieser Koagula während der vor Beendigung der Agonie begonnenen Untersuchung[1]) zunahm. So konnten sich in der zuerst untersuchten Lunge keine Koagula finden, während sie sich in der zuletzt untersuchten Lunge fanden; oder beim ersten Einschnitt in die Lunge fanden sich noch keine makroskopisch sichtbaren Thromben, wohl aber bei dem zweiten oder dritten Einschnitt. Es handelt sich hier offenbar um eine agonal erfolgende Ausdehnung von Lungenthromben.

Weiter fand sich in den Lungen nicht selten Ödem; doch war dieses gewöhnlich nicht sehr beträchtlich und nicht die Todesursache. In vielen Fällen fehlte Ödem. Es fehlte sehr häufig, falls der Tod in den ersten 2 oder 3 Minuten eintrat, und fand sich zuweilen in Versuchen, in denen das Tier längere Zeit am Leben blieb und durch Chloroform getötet wurde.

Zuweilen fanden sich auch Hämorrhagien in der Lunge, und es kam vor, daß sie die typische Form eines Infarktes aufwiesen. Auch diese konnten sich in Fällen finden, in denen das Tier erst beträchtliche Zeit nach der Injektion starb.

Es ist nun sehr wohl möglich, wenn wir uns auch mit Sicherheit darüber nicht aussprechen können, daß Ödem der Lunge etwas häufiger vorgefunden wurde, in Fällen, in denen die Einflußgeschwindigkeit des Serums 4 ccm, als in denen, in welchen sie nur 2 ccm pro Minute betrug.

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[1]) Zur Zeit der Vornahme der Autopsie waren die von der Cornea und Haut ausgehenden Reflexe erloschen.

Bei der mikroskopischen Untersuchung der Lunge ergab sich als der bei weitem häufigste Befund Hämorrhagien um die Pulmonalarterienzweige. In einigen Fällen konnte man sehen, wie das Blut in die Arterienwand eindrang. Weiterhin füllte das Blut benachbarte Alveolen.

Zuweilen schloß sich an diese Blutungen Ödem in den umliegenden Alveolen an. Wie auch in andern Fällen von Ödem, führte das Eindringen von Flüssigkeit in die Alveolen zu einer Ablösung der Alveolarepithelien in einigen Alveolen.

Worauf die periarteriellen Blutungen beruhen, läßt sich nicht mit Sicherheit feststellen. Sie sind unabhängig von Thrombenbildung; sie fanden sich z. B. auch in einem Falle, wo das Tier nach Injektion von 10 ccm einer 4 prozentigen Natriumzitratlösung in die Ohrvene starb. Es handelt sich vielleicht um starke Kontraktion der Lungenarterien mit Erhöhung des Blutdrucks und um folgende Zerreißung der Arterienwand.

In einem Falle, wo Schnitte durch einen Pulmonalarterienthrombus erhalten wurden, waren die Lungenkapillaren des zugehörigen Gebietes sowie auch die Lungenvenen stark gefüllt; es fanden sich hier auch bedeutende Ansammlungen von Leukozyten in den Gefäßen. Auch in andern Fällen fanden sich ähnliche Befunde. Es handelt sich hier wohl um eine Rückstauung des Blutes.

Das Blut, das während der Autopsie entnommen wurde, zeigte bald beschleunigte Gerinnung, in andern Fällen war die Gerinnung verlangsamt. Auch B o g g s [1]) fand keine Regelmäßigkeit nach intravenöser Injektion von artfremdem Serum. Doch lassen sich gewisse Regeln aufstellen, falls man eine größere Anzahl von Versuchen anstellt. In denjenigen Fällen, in denen das Blut in dem Herzen während der Agonie gerann, gerann auch das Blut in vitro sehr schnell, falls es frühzeitig entnommen wurde. In Fällen, in denen intrakardial Gerinnsel sich nicht bildeten, war häufig, aber nicht immer, eine verlangsamte Blutgerinnung in vitro zu beobachten.

Bei weiteren Versuchen dürfte sich vielleicht auch die Zeit, welche zwischen dem Tode und der Blutentnahme verfloß, als ein wichtiger Faktor erweisen, der mitentscheidet, ob man das Blut in der positiven oder negativen Phase vorfindet. Wie in vitro, so gerinnt auch in dem Körper das Blut nach einer gewissen Zeit, auch in solchen Fällen, in denen der Tod später als 9 Minuten nach Beendigung der Seruminjektion stattgefunden hat. Findet die Autopsie z. B. erst eine Stunde nach dem Tode statt, so findet man jetzt auch in dem Herzen Gerinnsel auch in solchen Fällen, in denen während der Agonie keine Gerinnsel sich gebildet hatten.

Als eine Folge der Injektion von Hundeserum findet in dem Körper Hämolyse statt. Falls man das Blut nach der Entnahme sofort zentrifugiert, findet man das Serum mehr oder weniger rot gefärbt. Auch durch Zählung der Erythrozyten vor und nach der Seruminjektion läßt sich diese Hämolyse feststellen. So sank z. B. in einem Versuche die Zahl der roten Blutkörperchen von 6 220 000 auf

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[1]) T. R. B o g g s , Über Beeinflussung der Gerinnungszeit des Blutes im lebenden Organismus. D. Arch. f. klin. Med. Bd. 79, 1904.

5 020 000; dies entspricht etwa einem Verlust von 19% der Erythrozyten im Verlaufe von ungefähr 6 Minuten. Das Kaninchen wog 1900 g. 10 ccm Serum wurden intravenös injiziert; das Tier starb 19 Minuten nach der Injektion; die erste Blutentnahme zum Zwecke der Erythrozytenzählung fand direkt vor der Injektion, die zweite Blutentnahme 5½ Minuten später statt.

Injektion einer Kombination von defibriniertem Kaninchenblut und Hundeserum. In 11 Versuchen wurde an Stelle von Hundeserum eine Kombination von defibriniertem Kaninchenblut und Hundeserum injiziert. Defibriniertes Kaninchenblut allein wird anstandslos vertragen, falls es eine Stunde nach der Blutentnahme oder später injiziert wird. In Kombination mit Hundeserum wirkt es tötlich. In unsern Versuchen wurde gewöhnlich zuerst das Kaninchenblut und sodann das Hundeserum injiziert. Bemerkenswert ist in diesen Versuchen, daß in zwei Fällen die Tiere schon nach Injektion von 5 und 6 ccm Hundeserum in wenigen Minuten starben; der Injektion des Hundeserums war in diesen Fällen eine Injektion von 7 bzw. 8 ccm Kaninchenblut vorangegangen. Möglicherweise macht die Einspritzung von Kaninchenblut das Hundeserum ein wenig gefährlicher für das Kaninchen; doch ist der Unterschied jedenfalls nur sehr gering. Ferner ist auffallend, daß in drei Versuchen, in denen die Tiere 4 Minuten nach der (getrennten) Injektion von Kaninchenblut und Hundeserum starben, sich keine Thromben im Herzen fanden, was der Fall gewesen wäre, falls zu dieser Zeit der Tod nach Injektion von Hundeserum allein stattgefunden hätte. Zugleich zeigen diese letztgenannten Versuche, wie variabel die Gerinnungszeit des Blutes unter diesen Bedingungen ist.

In den drei Experimenten erfolgte jedesmal der Tod 4 Minuten nach Beendigung der Injektion, die Gerinnungszeit des sofort nach dem Tode entnommenen Blutes war in dem zweiten Versuch 10 Minuten, in dem dritten 4 Minuten und in dem ersten Versuch hatte nach 30 Minuten nur eine teilweise Gerinnung stattgefunden.

Es ist wahrscheinlich, daß, falls eine Kombination von defibriniertem Kaninchenblut und von Hundeserum injiziert wird, die in dem Kaninchenblut nach dem Defibrinieren enthaltenen gerinnungbeschleunigenden Substanzen nicht ohne Bedeutung bleiben und in Verbindung mit dem Hundeserum den Tod herbeiführen.

2. Injektion von Hirudin und Hundeserum.

In der ersten Versuchsreihe, in der wir unverändertes Hundeserum injizierten, spielten Thromben im Herzen und in den Lungen eine bedeutende Rolle. Unter den Symptomen war die Dyspnoe, an die sich Konvulsionen anschlossen, am meisten in die Augen fallend. Diese Tatsachen legten den Gedanken nahe, daß intravaskuläre Gerinnungsvorgänge für die letale Wirkung des Hundeserums verantwortlich sein möchten. Es war nun möglich, diese Annahme einer experimentellen Prüfung zu unterziehen. Eingriffe, die die intravaskuläre Gerinnung aufheben oder vermindern, sollten dann die letale Dose des Hundeserums herabsetzen.

Intravenöse Injektion von Hirudin erschwert die Blutgerinnung. Falls also intravaskuläre Gerinnungsvorgänge allein oder in Verbindung mit andern Faktoren bei der letalen Wirkung des Hundeserums in Betracht kommen, sollte eine Kombination von Hirudin und Blutserum die letale Dose des Hundeserums erhöhen. Hierbei ist nun zu berücksichtigen, daß die gerinnungshemmende Kraft des Hirudins beschränkt ist. Hirudin wirkt sehr kräftig gegen Thrombin, es hemmt daher die gerinnungsbeschleunigende Wirkung des Hundeserums gegenüber dem Blutkörperchenfreien Plasma sehr wesentlich; Hirudin wirkt aber viel schwächer hemmend gegenüber den in den Geweben vorhandenen Gewebskoagulinen und ebenso gegenüber gewissen Bestandteilen der Erythrozyten, die bei der durch Hundeserum bewirkten Hämolyse frei werden.

Daher kann Hirudin, falls innerhalb der Gefäße eine stärkere Hämolyse durch Injektion von Blutserum des Hundes bewirkt wird, nur bis zu einem gewissen beschränkten Grade lebensrettend wirken.

Es wurden nun 26 Kaninchen mit Hirudin und Hundeserum injiziert. Die angewandten Hirudinmengen schwankten in den einzelnen Versuchen zwischen 80 und 20 mg; in der Mehrzahl der Versuche wurden 50 mg injiziert, einmal sogar nur 15 mg. Dabei kommen nun noch Variationen in der Stärke des Hirudins hinzu. Manchmal wurde zuerst das Hirudin in etwa 0,85 prozentigem NaCl gelöst, intravenös injiziert und ½ bis 2 Minuten später das Serum, in andern Fällen wurde das Hirudin oder ein größerer Teil desselben mit dem Serum gemischt eingespritzt; in einigen Fällen wurden nach Injektion dieser Mischung nach Ablauf einiger Minuten weitere, gewöhnlich kleinere Mengen Hirudin injiziert.

Das Resultat war nun, daß in der Mehrzahl der Versuche das Hirudin die letale Dose des Hundeserums erhöhte, und zwar schwankte in den einzelnen Versuchen die Erhöhung der Dose zwischen 50 und 100 %.

Wurde die Dose des Serums noch weiter erhöht, so blieben die Tiere nicht am Leben, aber sie starben weniger schnell als die Kontrolltiere. Auch die Symptome waren ähnlich, erst Beschleunigung der Atmung und vor dem Tode Konvulsionen. Doch konnten auch die Konvulsionen fehlen. Bei der Autopsie fanden sich nach einer vorhergehenden Hirudininjektion niemals Gerinnsel im Herzen oder in den großen Venen; wohl aber fanden sich Thromben in den Lungengefäßen in einer Anzahl der Fälle. Auch hier schienen diese erst agonal zu entstehen oder jedenfalls agonal sich auszudehnen, wie wir dies oben beschrieben haben bei Tieren, die Hundeserum ohne Hirudin erhalten hatten. Ödem der Lunge fand sich häufig in geringerem oder stärkerem Grade; doch konnte es auch fehlen. Bei der mikroskopischen Untersuchung fanden sich wieder Blutungen um die Pulmonalarterie. Das nach dem Tod entnommene Blut blieb längere oder kürzere Zeit ungeronnen.

Es wurden nun hier wiederum einige Erythrozytenzählungen vor und nach der Einspritzung des Hundeserums vorgenommen, um den Verlauf der intravaskulären Hämolyse zu ermitteln.

Es ergab sich in einem Versuch, daß vor der Injektion von 10 ccm Serum plus 50 mg Hirudin die Anzahl der Erythrozyten 6 750 000 betrug, 20 Minuten nach der Injektion war dieselbe auf 4 380 000 (Hämoglobin im Urin), 3½ Stunden nach der Injektion auf 3 860 000, 5½ Stunden nach der Injektion auf 3 576 000 gesunken.

Dieser Versuch zeigt, daß kurz nach der Injektion eine rasche, dann stundenlang eine langsamere Zerstörung der Erythrozyten stattfindet. Das Tier war während dieser Zeit schwach, aber blieb am Leben.

In einem andern Versuche hingegen starb das Tier allmählich unter zunehmender Schwäche.

In diesem Falle wurden 16 ccm Serum + 30 mg Hirudin injiziert. Schon vor der Injektion war die Zahl der Erythrozyten sehr niedrig, nämlich 3 760 000. Das Tier war also anämisch. 11 Minuten nach der Injektion war die Zahl auf 2 200 000 und 2 Stunden später auf 1 900 000 gesunken. Also auch hier eine ähnliche Kurve. Das Tier starb unter zunehmender Schwäche nach 4 Stunden.

Es mögen einige Beispiele für die Wirkung des Hirudins angeführt werden.

Serum 16 ohne Hirudin zeigte die folgende Wirkung: 7 ccm töteten in 9 Minuten, 7,2 ccm in 6 Minuten, 7,8 ccm in 2 Minuten, 8,6 ccm in 3½ Minuten.

Es wurden nun einem Kaninchen 80 mg Hirudin, gelöst in 7 ccm 0,85 prozentigem NaCl, injiziert. Eine halbe Minute später wurden 12,5 ccm Serum injiziert. Das Tier blieb am Leben. In ein anderes Tier wurden nach vorausgehender Injektion der gleichen Dose von Hirudin 10,5 ccm Serum eingespritzt. Hier blieb das Tier nicht am Leben, sondern starb nach 19 Minuten, also unter starker Verzögerung. Das Tier zeigte bald nach der Injektion Schwäche und nach 2½ Minuten schnelle Respiration. Darauf schien es sich zu erholen. Aber etwa 18 Minuten nach der Injektion traten plötzlich Konvulsionen auf, und dann starb das Kaninchen. In den Lungen fanden sich geringes Ödem sowie Thromben. Im Herzen waren Koagula nicht vorhanden. Das nach dem Tode entnommene Blut blieb in vitro lange flüssig.

In einem mit einem andern Serum angestellten Versuch starb das Kontrolltier nach Injektion von 8,3 ccm Serum in 2 Minuten. Ein Tier hingegen, das 15 ccm + 30 mg (sehr wirksamen) Hirudins erhalten hatte, blieb am Leben.

Ferner sei ein Versuch angeführt, in dem Injektion von Hirudin ohne Wirkung blieb.

Kontrolltiere starben nach Injektion von 10 ccm in 4 bis 5 Minuten. Kaninchen, die vor der Seruminjektion 30 bis 45 mg Hirudin erhielten, starben nach der Injektion von 10,5 bis 11,3 ccm Serum ungefähr wie die Kontrolltiere nach 3½ bis 5 Minuten, ebenfalls unter Konvulsionen.

In diesen drei Versuchen fand sich einmal kein Lungenödem, einmal nur geringfügiges und einmal ausgeprägtes Ödem. Thromben fanden sich in den Lungen, aber nicht in dem Herzen. In einem Falle, in dem die Autopsie bald nach Beginn der agonalen Konvulsionen gemacht wurde, ließ sich feststellen, daß die Thromben erst während der Agonie sich bildeten.

Im allgemeinen zeigte sich das Hirudin wirksam in den Fällen, in denen eine genügende Menge nicht zu stark abgeschwächten Hirudins injiziert wurde. Es waren besonders einige Versuche, in denen geringe Mengen von Hirudin gleichzeitig mit dem Serum anstatt vor dem Serum eingespritzt wurden, in denen Hirudin die letale Dose nicht erhöhte. Daß nun Hirudin eine solche distinkte Wirkung ausübte, beweist, daß Gerinnungsvorgänge im Kreislauf für den Tod nach Injektion von Hundeserum verantwortlich sind. Wahrscheinlich beruht aber die primäre

Schwäche und Respirationsbeschleunigung nach Injektion des Serums (die inner-halb der ersten 3 Minuten ablaufenden Symptome im Falle nicht letaler Wirkung) auf zentraler Wirkung des Serums, etwa auf Beeinflussung des vasomotorischen und Respirationszentrums. Es bleibt nun noch zu erklären, daß Hirudin die letale Wirkung des Serums nicht ganz aufhebt, sondern nur vermindert. Das beruht in erster Linie wohl darauf, daß, falls starke Hämolyse stattfindet, Hirudin, das ja nur für eine beschränkte Zeit im Blutgefäßsystem wirksam ist, nicht die Bildung kleiner Koagula verhindern kann, die sich dann in den Lungengefäßen ansammeln und diese verstopfen. Es findet daher nur eine gewisse Verzögerung des Todes statt. Wir finden daher später Zeichen von in den Lungen sich ausdehnenden Thromben. Doch sind nicht die großen, mit dem Auge sichtbaren Thromben in der Lunge die direkte Todesursache, da diese sich ja wahrscheinlich erst nach Beginn der agonalen Symptome bilden, sondern es handelt sich offenbar primär um kleine Koagula, und die größeren Koagula stellen nur eine Ausdehnung der kleineren dar. Auch die Herzkoagula spielen bei der Serumwirkung keine Rolle, sie sind agonal; in keinem Falle fanden sich Herzthromben nach Injektion von Hirudin, auch in den Versuchen, in welchen eine Erhöhung der letalen Dose durch Hirudin nicht bewirkt worden war. Wir können aber auch nicht ausschließen, daß in einigen Fällen der Tod nach der Injektion von Hirudin und Serum durch Lungenödem, in andern Fällen durch starke Hämolyse in schwachen Tieren und durch direkte oder indirekte zentrale Wirkung auf gewisse Teile der Medulla oblongata und des Rückenmarks zustande kam. Jedenfalls beweisen diese Ver-suche, daß die primäre Todesursache nach intravenöser Injektion genügender Mengen von Hundeserum in Kaninchen auf intravitalen Gerinnungen und Ver-stopfung vieler kleiner Lungengefäße beruht.

Wir berücksichtigen aber, wie ausdrücklich bemerkt werden soll, in diesen Versuchen nur die Fälle, in denen der Tod innerhalb der ersten Stunden nach der Injektion und nicht erst später eintrat. Über die Ursachen des später eintretenden Todes haben wir keine Untersuchungen angestellt.

3. Einfluß der Wärme auf die letale Dose des Hundeserums.

Zur weiteren Analyse der toxischen Wirkung des Hundeserums war es von Interesse, den Einfluß der Erwärmung festzustellen.

Es ergab sich, daß Erwärmen auf 56° während einer halben Stunde die Giftigkeit des Serums aufhebt.

So wurde z. B. die Injektion von 16 ccm ohne Symptome ertragen. Blutserum, das 23 Stunden im Thermostat (38°) gehalten wurde, hatte beträchtlich an Toxizität verloren. Nach Injektion von 10,7 ccm und 16,1 ccm traten nur geringe Reaktionen auf, und die Tiere erholten sich.

Auch Serum, das 4½ Stunden bei 38° im Thermostat gestanden hatte, hatte an Kraft ver-loren. Während 12 ccm Kontrollserum (8 Tage alt, daher wohl ein wenig getrübt, obwohl im Eisschrank gehalten) in 7 Minuten töteten, erholt sich ein Kaninchen nach Injektion von 23 ccm desselben Serums, nachdem es 4½ Stunden bei 38° gehalten worden war. Doch stirbt ein zweites Tier nach Injektion von 23,6 ccm eines solchen Serums; hier fanden sich Koagula im rechten Herzen.

Schon ein Aufenthalt von 1¼ Stunden bei 38° im Thermostat schwächt das Serum in nachweisbarer Weise.

Während 9,2 ccm Kontrollserum ein Kaninchen in 6 Minuten und 6 ccm in 2½ Stunden töteten, töteten nach 1¼stündiger Erwärmung auf 38° 9,2 ccm in 15 Minuten, 16,6 ccm in 2 Minuten (Koagulum im rechten Herzen), 27,7 ccm direkt nach Abschluß der Injektion (Koagulum im rechten Herzen und in den Venen). Nach Injektion von 6,3 ccm erwärmten Serums erholte sich ein Tier.

Serum, das 1¾ Stunden bei Zimmertemperatur stand, war nicht merklich geschwächt.

Ein solches Serum tötete in einer Dose von 8,5 ccm sofort mit Koagulis in Herzen und Venen, während Kontrollserum in einer Dose von 8,3 ccm in 2 Minuten und in einer Dose von 9 ccm in 5 Minuten tötete. Nach Injektion von 6 ccm erholte sich ein Tier.

Wir sehen also, daß Erwärmen auf 56° während einer halben Stunde das Serum unschädlich macht, daß eine Erwärmung auf 38° während einer Periode von 2 bis 23 Stunden das Serum nachweisbar schwächt, wobei längere Erwärmung wirksamer ist; daß aber etwa zweistündiges Stehen bei Zimmertemperatur eine merkliche Abnahme in der letalen Wirkung des Serums nicht bewirkt.

4. Adsorptionsversuche.

49 Kaninchen wurden mit Hundeserum injiziert, das mit fein zerteilten Organen des Hundes oder des Kaninchens während 1 oder 2 Stunden im Schüttelapparat vermischt worden war. Die Proportionen, in denen Serum und Organe gemischt wurden, waren verschieden in verschiedenen Versuchen. Es wurden in der Mehrzahl der Versuche 23 oder 13 g des fein zerteilten Organes mit 100 ccm Serum gemischt. Vor dem Gebrauch wurden die Suspensionen durch mehrere Lagen von engmaschigem Tuch filtriert und dann mehrere Male zentrifugiert. Die benutzten Organe waren: Kaninchen- und Hundehirn, Kaninchenniere und Hunde- und Kaninchenleber. Es ergab sich nun, daß durch Adsorption ein Teil der schädlichen Substanzen aus dem Serum entfernt werden. Die letale Dosis wird erhöht, und zwar in den verschiedenen Versuchen verschieden stark. Ein durchgreifender Unterschied in der Wirkung der verschiedenen Organe bestand nicht. Ein Unterschied in der Wirkung des so behandelten Serums von dem gewöhnlichen Serum lag darin, daß in einer Anzahl von Versuchen, in denen die Tiere innerhalb von 6 Minuten nach beendigter Injektion starben, sich im Herzen keine Koagula fanden. Führt Injektion von unbehandeltem Serum in dieser Zeit zum Tode, so finden sich regelmäßig Herzkoagula. Doch waren in einigen Fällen, in denen das Tier in wenigen Minuten starb, Herzkoagula, trotz vorheriger Behandlung des Serums mit Organen, vorhanden. Diese Verschiedenheit in dem Verhalten der beiden Sera ist entweder dadurch verursacht, daß nach Einwirkung der Organsuspension gewisse Substanzen, die aus den Geweben extrahiert werden, die Eigenschaften des Serums ändern, oder möglicherweise hängt dieses Verhalten davon ab, daß zuweilen trotz sorgfältiger Behandlung kleine Partikel von Organsuspension in dem Serum blieben und Lungenembolien verursachten.

Während, wie wir früher sahen, das Blut des Kaninchens nach Injektion einer Dose von Hundeserum, die den Tod innerhalb weniger Minuten herbeiführt, gewöhnlich sehr schnell in vitro gerinnt, falls es nicht zu spät entnommen wird, gerinnt das Blut, das Kaninchen entnommen wird, die innerhalb weniger Minuten nach Injektion von mit Organsuspension behandeltem Serum ohne Bildung von Koagula im Herzen starben, gewöhnlich langsam; es begann aber schnell in Fällen, in denen sich nach Injektion solchen Serums intrakardiale Koagula vorfanden. In Tieren, die später starben, war meist die Blutgerinnung in vitro verzögert.

Die Symptome, die sich nach der Injektion dieses Serums entwickelten, waren ähnlich wie nach Injektion gewöhnlichen Hundeserums; Schwäche und Konvulsionen waren häufig vor dem Tode vorhanden. Bei der Autopsie wurden zuweilen makroskopisch Gerinnsel in Lungengefäßen gefunden.

In einem Versuch, in dem das Kontrollserum in einer Dose von 8,6 ccm das Kaninchen in 1½ Minuten tötete, blieb ein Tier nach Injektion von 9½ ccm mit Kaninchenhirn behandelten Serums am Leben; ebenso nach Injektion von 10 ccm mit Hundehirn oder von 12 ccm mit Kaninchenniere behandelten Serums.

Aber ein Tier, das eine Injektion von 15 ccm mit Hundehirn behandelten Serums erhielt, starb nach 3½ Minuten, ohne daß sich Herzthromben fanden.

In einem andern Fall, in dem 10 ccm unbehandelten Serums in 5 Minuten töteten, blieb ein Tier, das mit 13 ccm mit Hundehirn behandelten Serums injiziert wurde, am Leben; ein anderes Kaninchen starb nach Injektion von 18 ccm mit Kaninchenhirn behandelten Serums erst nach 42 Minuten; 18 ccm mit Kaninchenleber behandelten Serums wurden gut ertragen, 19 ccm mit Hundeleber behandelten Serums töteten erst in 1 bis 2 Stunden.

5. Injektion von Hundeserum in immunisierte Kaninchen.

Durch wiederholte Einspritzung von Hundeserum in Kaninchen kann ihre Toleranz gegen intravenöse Injektion von Hundeserum sehr erhöht werden. In eine Anzahl Kaninchen wurde alle 3 bis 7 Tage Hundeserum intravenös injiziert in anfangs sehr langsam wachsender Dose; später wurde jedesmal eine um 3 ccm größere Dose injiziert. Nach etwas mehr als 2 Monaten konnten 40 ccm Hundeserum ohne schädliche Folgen injiziert werden; also etwa die sechs- bis siebenfach letale Dose. Auf der andern Seite können natürlich (wieder eine von uns vor etwa 4 bis 5 Jahren beobachtete) auch anaphylaktische Erscheinungen bei Kaninchen, die mit Hundeserum injiziert werden, beobachtet werden.

Die Immunisierung beruht aller Wahrscheinlichkeit darauf, daß in immunisierten Kaninchen eine Auflösung der Erythrozyten durch das Hundeserum nicht stattfindet.

II. Versuche mit Rinderserum.

1. Injektion von Rinderserum ohne weitere Zusätze.

In der Beschreibung dieser Versuche können wir uns viel kürzer fassen, da die Technik und die Ergebnisse in vieler Hinsicht den beim Hundeserum geschilderten gleichen. Doch bestehen einige charakteristische Unterschiede, und es wird im

wesentlichen darauf ankommen, diese hervorzuheben. Die Symptome nach In-
jektion von Rinderserum sind im wesentlichen dieselben wie nach Injektion von
Hundeserum: Schwäche, Atmungsbeschleunigung bald nach der Einspritzung; falls
der Tod in wenigen Minuten eintritt, schließen sich Unruhe und Konvulsionen an;
falls der Tod erst später eintritt, erholt sich das Tier im Anfang oder bleibt schwächer
und später wird die Schwäche sehr ausgesprochen, und das Tier stirbt unter Kon-
vulsionen. Doch können diese auch ausbleiben. Die Respiration hört vor der
Herzaktion auf. Nach dem Tode finden sich Erweiterung des rechten Herzens
und häufig Koagula in den Lungengefäßen; aber auch hier entstehen dieselben
(die Koagula) wahrscheinlich agonal, ebenso wie beim Hundeserum; die Ergebnisse
bei frühzeitiger Autopsie weisen darauf hin. Ein merklicher Unterschied zwischen
Rinder- und Hundeserum besteht darin, daß das erstere keine intrakardiale Ge-
rinnungen verursacht, auch wenn der Tod innerhalb der ersten Minuten nach der
Injektion eintritt. Ebensowenig finden sich Gerinnsel in den großen Venen. Nur
einmal fand sich ein Serum, das sich hierin ähnlich wie Hundeserum verhielt;
aber hier lag offenbar eine Anormalität vor. Entsprechend fanden wir auch häufiger
eine Verzögerung der Blutgerinnung in vitro als bei Hundeserum, auch in den
Fällen, in denen das Tier innerhalb der ersten Minuten starb. Doch wurde auch
in einem Falle, in dem das Blut sehr früh in der Agonie entnommen wurde, beob-
achtet, daß die Gerinnung beschleunigt war. Zählung der Erythrozyten vor und
nach der Injektion ergibt auch hier eine merkliche Verminderung der Blutkörper-
chen. Mikroskopisch zeigten die Lungen nicht selten Hämorrhagien um einige
Äste der Pulmonalarterie; ebenso wie auch zuweilen etwas Ödem, das in einigen
Fällen auch makroskopisch feststellbar war.

Die letale Dose des Rinderserums ist nicht sehr verschieden von der des Hunde-
serums, doch ist das erstere wohl um ein geringfügiges aktiver. Auch ist die Kurve,
welche die Zeit bis zum eintretenden Tod als Funktion der Menge des eingespritzten
Serums darstellt, ähnlich wie beim Hundeserum. Rinderserum, das 4 und 6 Tage
im Eisschrank gehalten wurde, hatte nicht wesentlich an Wirksamkeit verloren;
doch war in einem Falle nach 8 Tagen das Serum merklich schwächer geworden.

Im ganzen wurden 26 Kaninchen mit nicht vorbehandeltem Rinderserum
ohne weiteren Zusatz injiziert.

Einige Beispiele für die letale Dose seien angeführt:

In einem Falle töteten 7 ccm in 2 Minuten, 10 ccm in 1 Minute. Mit einem andern Serum
nach Injektion von 4,9 ccm: das Tier bleibt am Leben. 7½ ccm töteten in 6 Minuten. 10 ccm
in 2 Minuten. In einem andern Versuch: 5 ccm töten in 12 Minuten, 6 ccm in 4 Minuten. In
einem andern Falle töteten 5 ccm in 8½ Minuten, 8 ccm in 4 Minuten.

Also Rinderserum unterscheidet sich von Hundeserum in seiner Wirkung
auf Kaninchen 1. dadurch, daß die letale Dose für das erstere ein wenig geringer
ist, 2. daß es in der Regel im Gegensatz zum Hundeserum keine intrakardialen
Gerinnungen bewirkt, und 3., daß wohl im Zusammenhang mit dem zweiten Punkt
nach der Injektion die Gerinnung des Kaninchenblutes in vitro in einer größeren

Anzahl von Fällen verlangsamt ist. In allen andern wesentlichen Punkten gleichen sich Rinder- und Hundeserum in ihrer Wirkung auf Kaninchen; und das weist darauf hin, daß der Tod in beiden Fällen nicht in identischer, aber doch in verwandter Weise herbeigeführt wird.

2. Injektion von Hirudin und Rinderserum.

Alles, was über die Dose und Art der Einspritzung bei dem Hundeserum gesagt wurde, gilt auch für das Rinderserum.

Die Ergebnisse weichen aber im Falle des Rinderserums nicht unwesentlich von den mit Hundeserum erhaltenen ab. Während, wie wir sahen, im Falle des letzteren Hirudin eine deutliche Wirkung ausübt, indem es die letale Dose erhöht oder die Zeit bis zum Eintritt des Todes verlängert, ist Hirudin in Kombination mit Rinderserum wirkungslos; kleine Schwankungen, die beobachtet werden, liegen innerhalb der Grenzen der natürlichen Schwankungen nach Injektion von Serum ohne Zusatz. Auch mit Hirudin trat der Tod gewöhnlich nach Injektion von 5 bis 7 ccm Rinderserum pro Kilogramm Tier ein. Nur in einem Falle hatte Hirudin eine günstige Wirkung, und zwar in dem Falle, in dem das Rinderserum, entgegen dem gewöhnlichen Verhalten, intrakardiale Gerinnung hervorrief. Während in diesem Falle die Kontrolltiere nach Injektion von 7 und 9 ccm Rinderserum in wenigen Minuten starben, starb ein Tier nach Zusatz von Hirudin und Injektion von 7 ccm Serum erst nach einer Stunde, und ein anderes Tier erholte sich nach Injektion von 11 ccm. Aber in diesem Falle haben wir es, wie erwähnt, mit einem ungewöhnlichen Serum zu tun.

Ebenso wie die Tatsache, daß nach Injektion von Rinderserum intrakardiale Gerinnungen ausbleiben, weist auch dieser weitere Befund die Wirkung des Hirudins betreffend darauf hin, daß im Gegensatz zum Hundeserum intravaskuläre Blutgerinnungen im Falle des Rinderserums für die letale Wirkung des Serums nicht von wesentlicher Bedeutung sind. Trotzdem muß nach Injektion von Rinderserum die direkte Todesursache sehr ähnlich sein der nach Injektion von Hundeserum beobachteten. Darauf weist die Ähnlichkeit der Symptome und Autopsiebefunde in beiden Fällen hin.

Die Untersuchung der Wirkung der beiden Sera in vitro wird es uns ermöglichen, eine diesen Postulaten entsprechende Erklärung der Todesart im Falle der beiden Sera zu geben.

Die Symptome wurden durch die Beifügung oder vorherige Injektion von Hirudin nicht wesentlich verändert, ebensowenig die makroskopischen oder mikroskopischen Ergebnisse der Autopsie. In keinem Falle fanden sich Koagula im Herzen und nur in einem Falle sichtbare Thromben in den Lungengefäßen. In 8 Tieren fand sich kein Lungenödem, in 7 Fällen war solches in geringerem oder stärkerem Maße vorhanden. Auch die periarteriellen Hämorrhagien waren vorhanden.

Entsprechend den oft beträchtlichen Dosen von Hirudin blieb das Blut bei der Entnahme nach dem Tode des Tieres gewöhnlich eine Zeitlang flüssig; doch fanden sich hier beträchtliche Variationen; in einem Falle z. B. begann die Gerinnung nach 40 Minuten, in einem andern Falle nach 15 oder sogar nach 7 Minuten. Hierbei ist aber zu berücksichtigen, daß das Blut meist ohne Kanüle aufgefangen wurde, so daß also die Gewebskoaguline einwirken konnten, ferner, daß in den Gefäßen Hämolyse auftrat, welche die Gerinnung beschleunigte.

19 Kaninchen wurde eine Kombination von Hirudin und Rinderserum injiziert.

3. Einfluß des Erwärmens auf das Rinderserum.

In 5 Kaninchen wurde Rinderserum injiziert, das während einer halben Stunde auf 56⁰ erwärmt worden war. Dieses Serum wurde in großen Dosen ohne schädliche Wirkung vertragen. Also die den Tod herbeiführende Substanz wird auch hier ebenso wie beim Hundeserum durch ein halbstündiges Erwärmen auf 56⁰ zerstört.

7 Kaninchen wurden intravenös mit Rinderserum injiziert, das 2½ bis 4 Stunden bei 38⁰ im Thermostat gehalten worden war. Dieses Serum war gegenüber dem im Eisschrank gehaltenen nicht merklich abgeschwächt. Letzteres tötete z. B. in einem Versuch in einer Dose von 8 ccm in 4 Minuten, in einer Dose von 5 ccm in 8½ Minuten, während das auf 38⁰ erwärmte Serum in einer Dose von 10 ccm in 3 Minuten und in einer Dose von 6 ccm in 5 Minuten tötete.

Wo in diesen Fällen größere Mengen Serum injiziert wurden, starb das Tier direkt nach der Injektion mit Konvulsionen; in einem dieser Fälle war das Ergebnis der Autopsie negativ, in dem andern fand sich Lungenödem.

Trat der Tod erst nach längerer Zeit ein, so stellten sich 1 Minute nach der Injektion schnellere Respiration und Schwäche ein; nach 8 Minuten erholte sich das Tier, um nach 22 Minuten wieder sehr schwach zu werden und nach 24 Minuten zu sterben.

4. Injektion von Rinderserum, das vorher mit Organemulsionen behandelt worden war.

In bezug auf die Technik der Versuche gilt auch hier alles, was früher im Falle des Hundeserums gesagt wurde, nur daß in diesen Versuchen Kaninchenhirn, Rinderhirn und Rinderniere benutzt wurden. Falls eine genügende Menge Kaninchenhirn (7 bis 15 g auf 40 ccm Serum) benutzt wurde, wurde die letale Dose des Rinderserums merklich, nämlich etwa auf 16 bis 20 ccm, erhöht; wurde aber eine kleinere Menge Kaninchenhirn (3,5 g oder weniger auf 40 ccm Serum benutzt,) so war eine merkliche Adsorptionswirkung nicht vorhanden.

In diesen Versuchen wirkte nun das Rinderhirn deutlich schwächer adsorbierend als das Kaninchenhirn, nach Injektion von 8 bis 17 ccm von mit Rinderhirn behandeltem Serum starben die Kaninchen in 1 bis 3 Minuten. Wie im Falle des Hundeserums hatte die Injektion solchen (adsorbierten) Serums zur Folge, daß

das Blut des injizierten Kaninchens in der großen Mehrzahl der Versuche nach der Entnahme langsamer koagulierte als in Versuchen mit unbehandeltem Rinderserum. Über die möglichen Ursachen für diesen Befund gilt das beim Hundeserum Gesagte. Diese gerinnunghemmende Wirkung war auch in solchen Fällen vorhanden, in denen das Tier am Leben blieb. In einem Falle jedoch war eine Gerinnungsmöglichkeit in vitro nicht vorhanden.

Merkwürdigerweise fanden sich in einigen Fällen, in denen Rinderserum nach Vorbehandlung mit Rinderhirn den Tod herbeiführte, Blutkoagula im rechten Herzen. Wie weit hierfür die Behandlung mit Rinderhirn verantwortlich zu machen ist, müßte durch weitere Versuche entschieden werden. Im übrigen waren Symptome und Autopsiebefunde nach Injektion solchen Serums dieselben wie nach Injektion von nicht vorbehandeltem Rinderserum.

18 Kaninchen wurden mit solchem Serum injiziert.

III. Versuche in vitro.

Um weitere Aufschlüsse über die Wirkungsart des Hunde- und Rinderserums auf Kaninchenblut zu erhalten, war es nötig, die Einwirkung der Sera auf das Kaninchenblut in vitro zu prüfen und mit ihrer Wirkung nach intravenöser Injektion in das lebende Tier zu vergleichen.

Es war nun nicht möglich, die Verhältnisse in vitro denen in vivo ganz entsprechend zu gestalten. Dazu wäre es nötig gewesen, unverändertes Blut in vitro zu benutzen. Das ist natürlich nicht möglich. Außerdem ist zu berücksichtigen, daß nach Injektion von artfremdem Serum in vivo Reaktionen stattfinden, die sich in dem Auftreten der sogenannten negativen Gerinnungsphase[1]) äußern und die natürlich bei Versuchen in vitro wegfallen. Am besten schien es zu sein, die Gerinnung des Blutes durch ultravenöse Injektion einer geeigneten Quantität von Hirudin in das Kaninchen zu hemmen und dann dieses Blut durch eine Kanüle zu entziehen und in seinem Verhalten gegenüber verschiedenen Quantitäten von Hunde- und Rinderserum zu prüfen. Hierbei war es nötig, einen Überschuß von Hirudin, der das Blut völlig ungerinnbar gemacht haben würde, zu vermeiden. Dieses Verfahren erlaubte uns nicht nur, den Einfluß des Serums auf Hämolyse und Agglutination der Erythrozyten, sondern auch auf die Gerinnung des Plasmas zu prüfen. Sodann wurde auch gesondert an Stelle von Hirudinblut defibriniertes Blut des Kaninchens benutzt. Jeweilen wurden Parallelversuche mit Hunde- und Rinderserum angestellt, auch wurde der Einfluß der Wärme auf das Hunde- und Rinderserum in vitro untersucht.

Es wurden 5 solcher Versuchsreihen in vitro angestellt. Zu 1 ccm Kaninchenblut + Hirudin oder zu 1 ccm defibrinierten Kaninchenblutes wurden absteigende Mengen Serum (von 1 ccm bis 0,1 ccm) zugesetzt und die folgende Hämolyse, Agglutination und Koagulation beobachtet.

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[1]) Vgl. über die Vorgänge bei der Blutgerinnung die zusammenhängende Darstellung von Leo Loeb im Biochemischen Zentralblatt Bd. VI, 1907.

Die Ergebnisse waren wie folgt: R i n d e r s e r u m agglutiniert die Erythrozyten des Kaninchens viel stärker als Hundeserum. Die Flocken der agglutinierten Blutkörperchen werden bald sehr umfangreich und sinken zu Boden. Um diese am Boden liegende Masse agglutinierter Erythrozyten beginnt die Koagulation des Plasmas; von hier breitet sich die Koagulation in die überstehende Flüssigkeit aus. Die Hämolyse kann bei Zufügen von Rinderserum ebenso stark sein wie mit Hundeserum; die Koagulation kann sogar stärker sein. Mit abnehmenden Mengen von Rinderserum nimmt die Hämolyse und Agglutination ab; bis zu einer gewissen optimalen Serummenge kann zuweilen die Blutkoagulation sogar zunehmen, um dann bei weiterer Abnahme des Rinderserums wieder geringer zu werden. Ein halbstündiges Erwärmen des Serums auf 56° vermindert die Agglutination außerordentlich, ohne sie aber vollständig aufzuheben. Ein solches Rinderserum verursacht weniger Agglutination als ein normales oder auf 56° erwärmtes Hundeserum. Die Hämolyse und Blutkoagulation wird durch Zusatz von auf 56° erwärmtem Rinderserum nicht hervorgerufen (bzw. beschleunigt). 2½- bis 3-stündiges Verweilen des Serums bei 38° schwächte die agglutinierende und hämolytische Kraft des Rinderserums in geringem Maße; doch bleibt auch unter solchen Umständen die Agglutination noch stark. Fügt man in vitro noch mehr Hirudin hinzu, so bleibt die Agglutination unverändert, aber die Koagulation wird aufgehoben.

H u n d e s e r u m bewirkt geringe Agglutination, die nach Erwärmen auf 56° bestehen bleibt. Dasselbe bewirkt kräftige Hämolyse und Koagulation des Plasmas. Letztere beginnt an verschiedenen Stellen entlang der Wand des Reagenzglases und erstreckt sich bald diffus über das ganze Blut. Die kleinen agglutinierten Flocken werden auch hämolysiert. Erwärmen auf 56° hebt die Hämolyse und Koagulation auf. Mehrstündiges Stehen bei 38° im Thermostat schädigt die Hämolyse und Koagulation ein wenig; insbesondere die Hämolyse. Die Einwirkung suspendierter Organstücke verringert durch Adsorption die hämolytische Kraft des Hundeserums. Am günstigsten wirkte hierbei das Gehirn, und zwar erwies sich in einigen Versuchen das Kaninchenhirn stärker wirksam als Hundehirn. Die Leber war schwächer in ihrer Wirkung.

Wir sehen also, daß das Rinderserum vor allem ein agglutinierend wirkendes Serum ist, daß es viel stärker agglutinierend wirkt als das Hundserum. Letzteres ist stark hämolytisch wirksam. Diese beiden Wirkungen — die Agglutination im Falle des Rinderserums, die Hämolyse im Falle des Hundeserums — bleiben aus oder fast aus (letzteres im Falle der Agglutination durch Rinderserum) nach Erwärmen auf 56°. Ebenso wie hiernach bei intravenöser Injektion das Serum aufhört, schädlich zu sein. In bezug auf Blutgerinnung wirken in beiden Fällen vor allem die Stromata der Erythrozyten koagulierend. Im Falle des Rinderserums sind die Stromata agglutiniert und bilden bald einen großen Haufen, um den dann die Koagulation beginnt. Es handelt sich hier also infolge der agglutinativen Prozesse, die vorhergehen, um lokale Gerinnungsvorgänge, die sich erst

sekundär in die überstehende Flüssigkeit ausbreiten, welch letztere nur mehr eine relativ geringe Zahl von Stromata enthalten kann. Im Falle des Hundeserums sind die Stromata in sehr kleinen Haufen in dem ganzen Plasma suspendiert, und deshalb ist hier die Blutgerinnung diffus. Hirudin hat nur einen Einfluß auf Koagulation, aber nicht auf agglutinative Prozesse. Außer den Stromata der lädierten Erythrozyten kommen noch in dem Serum vorhandene, direkt gerinnung-wirkende Substanzen in Betracht [1]). Diese sind nun, soweit Hirnblut in Betracht kommt, viel wirksamer im Rinderserum als im Hundeserum. Diese Substanzen, die direkt gerinnungbeschleunigend wirken, mögen zur Ausbreitung der Gerinnung im Kaninchenblut nach Zusatz von Rinderserum beitragen. Es dürfte wohl nicht notwendig sein, die ausgedehnten Versuche im einzelnen anzuführen; es sei nur bemerkt, daß zur Gerinnung des Hirudinblutes 50 bis 70 mg Hirudin intravenös in ein Kaninchen injiziert wurden.

Zusammenfassung und Schlußfolgerungen.

Auf Grund der hier mitgeteilten Versuche ist es nun möglich, die Wirkungs-weise des Hunde -und Rinderserums nach intravenöser Injektion in das Kaninchen zu verstehen und die letale Wirkung derselben zu erklären. Alle diese auf ver-schiedenem Wege erhaltenen Resultate lassen sich in einheitlicher und wider-spruchsloser Weise zusammenfassen.

Das Hundeserum bewirkt nach der Injektion in dem Kaninchenblut beträcht-liche Hämolyse. Hierdurch werden in dem ganzen Blut verteilte gerinnung-erregende Substanzen frei, die an die Stromata der Erythrozyten gebunden sind. Diese letzteren agglutinieren nur in geringfügiger Weise; sie bleiben daher über das ganze Blut verteilt. Die agglutinierten Massen sind nicht groß genug, um die kleineren Lungenarterien zu verstopfen, und sie dürften daher die Lungenzirkulation nicht ernstlich erschweren. Wohl aber bewirken die Stromata die Bildung kleiner Fibringerinnsel in dem zirkulierenden Blute. Diese Gerinnsel werden in die Lungen-gefäße geführt und versperren hier die kleinen Äste der Lungenarterien und die Kapillaren und führen so den Tod unter Erstickungserscheinungen (Dyspnoe, Konvulsionen) herbei. Infolge dieser Widerstände im Lungenkreislauf findet eine Verlangsamung des Blutstroms im rechten Herzen und in den Körpervenen statt, und infolge davon ist das Blut in diesen Bezirken ein günstiges Substrat für die im Blut verteilten gerinnungbeschleunigenden Substanzen; in der Agonie finden daher, falls sehr viel gerinnungbeschleunigende, aus den Stromata der Erythro-zyten stammende Substanzen im Blute vorhanden sind, was der Fall sein wird, wenn relativ größere Serummengen injiziert wurden, zuerst Gerinnungen im rechten Herzen, weiterhin in den großen Körpervenen und in den ausgesprochensten Fällen auch im linken Herzen statt. Ferner schreitet während der Agonie die Gerinnung von den kleinen Ästen der Lungenarterie auf größere Lungengefäße fort, und so

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[1]) Hierüber sollen an anderer Stelle ausführlichere Mitteilungen gemacht werden.

werden während der Agonie Thromben in den großen Lymphgefäßen sichtbar. Die Erschwerung der Lungenzirkulation kann zu mehr oder weniger beträchtlichem Ödem, und in Verbindung mit dyspnöischen Atembewegungen zu periarteriellen Hämorrhagien führen. Wahrscheinlich ist die Dyspnoe allein hierzu schon genügend. Ob die verschiedene Verteilung von Kohlensäure und Sauerstoff im arteriellen und venösen Blut für die größere Leichtigkeit der Gerinnung in dem venösen Zirkulationsgebiet ebenfalls in Betracht kommt, mag dahingestellt bleiben. Es ist nicht unwahrscheinlich, daß die häufig in den ersten zwei Minuten nach der Injektion eintretende primäre Schwäche und Respirationsbeschleunigung auf Beeinflussung von Nervenzentren abhängt. Hiervon können sich aber die Tiere erholen.

Wird nun Hirudin in genügender Menge eingeführt, so wird hier auch die Bildung der Gerinnsel verringert. Ob das Hirudin auch erweiternd auf die kleineren Lungenarterien wirkt, muß dahingestellt bleiben. So wird durch Hirudin die Verstopfung der kleinen Pulmonalarterienäste bedeutend verringert, oder falls nur kleine Serummengen injiziert worden waren, fast ganz aufgehoben. Aber die Wirkung des Hirudins kann nur eine beschränkte sein, da das Hirudin große Mengen gerinnungbeschleunigender Substanz in vivo nicht neutralisieren kann; ferner findet in vivo bald eine Beseitigung von Hirudin statt. Doch genügen die Hirudinmengen immer, um die Koagulation in dem Herzen und in den großen Venen aufzuheben. Es ist nun auch möglich, daß, wenn größere Mengen Serum mit Hirudin injiziert werden, die Tiere infolge der beträchtlichen Hämolyse oder infolge der Wirkung des Serums auf das vasomotorische Zentrum und auf die respiratorischen Zentren sterben; doch liegen für eine solche Annahme keine direkten Beweise vor. Wird nun das Hundeserum auf 56° eine halbe Stunde erwärmt, so wird die hämolytische und Koagulation bewirkende Kraft des Serums aufgehoben, und infolgedessen wird das letztere unschädlich. Durch längeres Stehen bei 38° wird die hämolytische Kraft geschwächt und dadurch die letale Dose erhöht.

Durch Adsorption mit Organsuspensionen wird ein Teil der hämolytisch wirkenden Substanzen entzogen, dafür treten aber vielleicht andere Substanzen aus den Geweben in das Serum über und verändern das Serum, so daß jetzt nach der Injektion des Serums das dem Tiere entnommene Blut häufig langsamer in vitro gerinnt.

Infolge der wiederholten Injektion von Hundeserum bilden sich in dem Kaninchen antihämolytisch wirkende Substanzen; die Bildung von Gerinnseln wird daher in solchen Tieren erschwert, und dieselben können große Dosen von Hundeserum intravenös ertragen. Es soll jedoch das Blut solcher immunisierter Tiere noch weiterhin auf sein Verhalten gegenüber hämolytisch wirkenden und gerinnungbeschleunigenden Substanzen geprüft werden, und die hier gegebene Erklärung kann nur als eine vorläufige betrachtet werden, soweit sie sich auf die Widerstandsfähigkeit solcher immunisierter Tiere gegen Injektion von größeren Mengen von Serum bezieht.

Das R i n d e r s e r u m bewirkt nach seiner Injektion in dem Blute des Kaninchens eine beträchtliche Agglutination der Erythrozyten. Diese agglutinierten Massen werden in die Lungengefäße geführt und bewirken hier bald eine Verstopfung einer großen Zahl der kleineren Äste der Pulmonalarterie und führen so zum Tod unter Erstickungserscheinungen, Erweiterung des rechten Herzens. In diesem Falle sind aber die gerinnungbewirkenden Stromata nicht diffus in dem zirkulierenden Blut und den Venen verteilt, sondern sie agglutinieren sehr schnell zu größeren Haufen und bleiben in den Lungengefäßen stecken. Hier findet deshalb keine Massengerinnung des Blutes im rechten Herzen und in den Venen statt. Wohl aber bilden sich lokal Gerinnsel um die agglutinierten Haufen von Erythrozyten, die in den Lungengefäßen stecken; so kann sich dann agonal oder in andern Fällen vielleicht auch schon vorher die Bildung größerer Lungenthromben sekundär an die Verstopfung der Lungengefäße durch agglutinierte Zellhaufen anschließen. Falls der Tod nicht in den ersten Minuten eintritt, mögen dann hier sowohl wie nach Injektion des Hundeserums in gewissen Organen des Kaninchens sekundäre Reaktionen eintreten, die die Blutgerinnung erschweren und die Massenkoagulation des Blutes im rechten Herzen und in den großen Venen verhindern. Einspritzung des Hirudins ist ohne wesentliche Wirkung im Falle des Rinderserums, da Hirudin die Agglutination der Erythrozyten nicht verringert. Nach Injektion des Hirudins wird daher ebensowohl wie vorher eine Verstopfung der Lungengefäße durch agglutinierte Zellmassen stattfinden. Erwärmen des Rinderserums auf 56° während einer halben Stunde verringert die agglutinierende Wirkung des Serums so bedeutend, daß dasselbe nicht mehr imstande ist, eine Verstopfung der Lungengefäße herbeizuführen; es wird daher anstandslos ertragen. Hingegen ist ein mehrstündiges Stehen bei 38° nicht imstande, die agglutinierende Wirkung des Serums so weit herabzusetzen, daß der Tod dadurch hintangehalten wird.

Durch Adsorption der agglutinierenden Substanz durch gewisse Organsuspensionen wird ebenfalls die letale Dose des Rinderserums erhöht.

Falls der Tod nicht direkt im Anschluß an die Injektion, sondern erst etwas später eintritt, mögen vielleicht auch hier neben der Agglutination der Erythrozyten in gewissen Fällen eine bedeutende Zerstörung der Erythrozyten oder zentrale Wirkung auf das Nervensystem den letalen Ausgang herbeiführen.

Z u s a m m e n f a s s e n d   k ö n n e n   w i r   a l s o   f e s t s t e l l e n ,   d a ß im   w e s e n t l i c h e n   n a c h   i n t r a v e n ö s e r   I n j e k t i o n   v o n   a r t - f r e m d e m   S e r u m   d e r   T o d   e n t w e d e r   d u r c h   V e r s t o p f u n g d e r   L u n g e n g e f ä ß e   d u r c h   F i b r i n p f r ö p f e   o d e r   d u r c h   H a u f e n v o n   a g g l u t i n i e r t e n   E r y t h r o z y t e n   s t a t t f i n d e t   u n d   d a ß H u n d e s e r u m   u n d   R i n d e r s e r u m   z w e i   v e r s c h i e d e n e   T y p e n r e p r ä s e n t i e r e n :   d a s   H u n d e s e r u m   i s t   d e r   T y p u s   e i n e s h ä m o l y s i e r e n d   u n d   k o a g u l i e r e n d   w i r k e n d e n ,   d a s   R i n d e r - s e r u m   i s t   d e r   T y p u s   e i n e s   a g g l u t i n i e r e n d e n   S e r u m s . Hiermit soll nicht gesagt sein, daß die betreffenden Eigenschaften auf das eine

oder andere Serum beschränkt sind, sondern diese Abgrenzung soll die charakteristische Wirkung der beiden Sera zum Ausdruck bringen, diejenige Wirkung, durch welche sie den Tod der Tiere herbeiführen.

Die Natur des Problems bringt es mit sich, daß die Beweisführung eine indirekte sein mußte. Aber durch Untersuchung des Blutserums anderer Tiere nach den hier benutzten Methoden wird eine Prüfung der hier gewonnenen Ergebnisse möglich sein.

Zum Schlusse möge noch darauf hingewiesen werden, daß die hier erhobenen Befunde auch bei der Erklärung des Todes nach Injektion artfremder Erythrozyten von Interesse sein mögen. Ob sie gewisse Widersprüche, die sich bei derartigen Untersuchungen bisher ergeben haben, werden aufklären können, kann erst durch besondere Versuche entschieden werden[1]. Vielleicht müssen auch hier Kombinationen unterschieden werden, in denen die wesentliche Wirkung in Hämolyse der eingeführten Erythrozyten und in Koagulation besteht, und andererseits Kombinationen, in denen die Agglutination der fremden Erythrozyten vor allem in Betracht kommt.

[1] Vgl. A r t h u r F. C o c a , Die Ursache des plötzlichen Todes bei intravenöser Injektion artfremder Blutkörper. Virch. Arch. Bd. 196 1909 S. 92. Diese Arbeit erschien zu einer Zeit, da ein großer Teil unserer Versuche schon ausgeführt war.